REMARKS

I. Amendments

By this amendment, Claims 1, 3 and 15 have been amended and claim 2 has been canceled without prejudice to the filing of further continuing applications.

No change of inventorship is necessitated by this amendment.

II. Request for the Clarification of the Status of Claim 23

Claim 23 is an independent claim. Applicants cannot find any reason for the claim's rejection/objection in the Office Action, so assume that it may actually be allowable, although listed as objected to in the most recent Office Action Summary. Applicants respectfully request that the Examiner review and update the status of this pending claim.

III. Discussion of the 35 U.S.C. Sec. 112, First Paragraph Rejection of Claims 22 and 29

Claims 22 and 29 have been rejected under 35 U.S.C. Sec. 112, first paragraph as allegedly non-enabled as to allegedly requiring undue experimentation to practice the claimed invention. Applicants respectfully traverse this rejection, and aver that the specification does contain enough information for one skilled in the art to practice the methods of claims 22 and 29. Applicants' reasoning for the patentability of each of the two rejected claims is discussed separately below.

Claim 22 is Enabled

In claim 22, methods for treating several conditions with sustained release compositions of the compounds of SEQ. ID NO.: 1 are recited. To show how these methods of treating are supported by the specification, Applicants wish to direct the Examiner's attention to Example 7 and Experimental Example 4.

In Example 7 on page 47 of the specification, a compound of SEQ. ID NO.: 1 is referred to as peptide B. Peptide B is formulated into a sustained release composition, and tested. The results are described in Experimental Example 4, appearing on page 57. Table 4 of that example shows that the peptide B formulation caused suppression of testosterone.

GnRH agonists suppress testosterone secretion. The supplemental references in attached Appendix A indicate applications for GnRH agonists. Specifically, in the Robertson *et al.* reference, a gonadotrophin-releasing hormone agonist (ZOLADEX) is shown to be useful for the treatment of breast cancer. In the Filicori reference, a wide variety of clinical applications for gonadotrophin-releaseing hormone agonists are discussed. The recited prostate cancer (Sec. 4.5), prostatic hypertrophy (Sec. 4.11.1), endometriosis (Sec. 4.4), precocious puberty (Sec. 4.1), dysmenorrhea (Sec. 4.4) and breast cancer (sec. 4.6) are among them.

The specification shows the utility of the recited compounds for testosterone suppression. Those skilled in the art would understand that compounds which suppress testosterone are GnRH agonists. Those skilled in the art would also understand how GnRH agonists can be used to combat other diseases of the body, as illustrated by the supplemental references. Thus, Applicants find the Examiner's statement that "applicants have not even taken the first step, which is to show *in vitro* efficacy" to be incorrect.

Furthermore, dosage and administration information is provided on pages 41 and 42 of the specification.

Applicants believe that those skilled in the art given the teachings of their specification and understanding of GnRH agonists would be able to perform the recited methods without undue experimentation. Accordingly, claim 22 is enabled.

In addition, it appears that the Examiner is troubled by the term "cancer" in claim 22. In providing a number of references, the Examiner hopes to show that attempts to treat cancer may not be successful, concluding that since cancer treatment is unpredictable, undue experimentation would be required to practice the claimed invention. However, in claim 22 the Applicants do

not purport to treat cancer in general, but rather two specific forms of cancer: prostatic cancer and breast cancer. Should the Examiner wish to maintain the rejection, Applicants request that the Examiner support his position with references which are directed to the forms of cancer which their compounds treat.

Claim 29 is Enabled

For the relationship of the compounds of SEQ ID NO.:1 to GnRH agonists as supported with experimental evidence, the Examiner is referred to the preceding paragraphs. LH-RH agonists are disclosed on page 8, lines 6-15 of the specification. The compound of Example 7 falls under this definition.

In attached Appendix B, two references are provided which support claim 29. In the Pike *et al.* article, GnRH agonists are cited as contraceptives; and in the Fraser *et al.* article it is indicated that LH-RH agonists may be used for contraception.

Applicants believe that those skilled in the art given the teachings of their specification and understanding of GnRH agonists and LH-RH agonists would be able to use the recited sustained release compositions for contraception without undue experimentation. Accordingly, claim 29 is enabled.

Therefore Applicants respectfully request withdrawal of the 35 U.S.C. Sec. 112, first paragraph rejection of claims 22 and 29.

IV. Discussion of the 35 U.S.C. Sec. 112, Second Paragraph Rejection of Claims 7 and 8

Claims 7 and 8 have been rejected under 35 U.S.C. Sec. 112, second paragraph as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Specifically the Office Action stated that claims 7 and 8 have been objected to as allegedly not properly sub-generic to claim 6.

The Examiner believes that claims 7 and 8 are not properly sub-generic to claim 6. However, this is not the case. Lactic acid-glycolic acid polymers are defined in the specification

at page 12, lines 6-24. According to the specification, and as is understood by those skilled in the art, *homopolymers* of either lactic acid or glycolic acid are included in the broadly defined "lactic acid-glycolic acid" polymers, as the Applicants stated in their previous response.

Applicants apologize for having assumed that the Examiner understood the meaning of the term "homopolymer". Homopolymers are made by the connecting together of monomers of just one type, as indicated by the "homo" prefix. That is to say, that when the Applicants indicate in their specification that lactic acid-glycolic acid polymers include homopolymers and copolymers, it means that not only poly(lactic acid/glycolic acid) polymers are useful, but also that poly(lactic acid) and poly(glycolic acid) are other useful polymers. Therefore, when poly(lactic acid) - the *homopolymer* - is the intended lactic acid-glycolic acid polymer, there will be no glycolic acid. For this reason, the option recited claim 7 of having 100% lactic acid and no glycolic acid and the recited 100% lactic acid and no glycolic acid in claim 8 (equivalent to "the case where glycolic acid is absent" that the Examiner has a concern about) is properly within the scope of claim 6.

Therefore, Applicants assert that claims 7 and 8 are properly sub-generic to claim 6 and so respectfully request withdrawal of the 35 U.S.C. Sec. 112, second paragraph rejection.

V. Discussion of the 35 U.S.C. Sec. 103 Rejection over Sachs *et al.*

Claims 1, 4 and 15 have been rejected under 35 U.S.C. Sec. 103 as allegedly obvious in light of Sachs *et al.*, U.S. Patent No. 6,132,768. Applicants respectfully traverse the rejection.

By this amendment, the limitations of claim 2 have been incorporated into claim 1. Claim 2 has not been subjected to the present rejection. Applicants submit that their invention as set forth in claim 1 as amended is unobvious over the cited reference.

Claims 4 and 15 depend upon claim 1. Applicants submit that the more specific dependent claims are also non-obvious.

Therefore Applicants respectfully request withdrawal of the 35 U.S.C. Sec. 103 rejection over Sachs *et al.*

VI. Discussion of the 35 U.S.C. Sec. 103 Rejection over Palmer

Claims 1 and 15 have been rejected under 35 U.S.C. Sec. 103 as allegedly obvious in view of Palmer, U.S. Patent No. 5,270,305. Applicants respectfully traverse the rejection.

By this amendment, the limitations of claim 2 have been incorporated into claim 1. Claim 2 has not been subjected to the present rejection. Applicants submit that their invention as set forth in claim 1 as amended is unobvious over the cited reference.

Claim 15 depends upon claim 1. Applicants submit that the more specific dependent claim is also non-obvious.

Therefore, Applicants respectfully request withdrawal of the 35 U.S.C. Sec. 103 rejection over Palmer.

VII. Discussion of the 35 U.S.C. Sec. 103 Rejection over Wong *et al.*'097

Claims 1 and 15 have been rejected under 35 U.S.C. Sec. 103 as allegedly obvious in view of Wong *et al.*, U.S. Patent No. 5,869,097. Applicants respectfully traverse the rejection.

By this amendment, the limitations of claim 2 have been incorporated into claim 1. Claim 2 has not been subjected to the present rejection. Applicants submit that their invention as set forth in claim 1 as amended is unobvious over the cited reference.

Claim 15 depends upon claim 1. Applicants submit that the more specific dependent claim is also non-obvious.

Therefore, Applicants respectfully request withdrawal of the 35 U.S.C. Sec. 103 rejection over Wong *et al.* '097.

VIII. Discussion of the 35 U.S.C. Sec. 103 Rejection over Wong *et al.* '194

Claims 1, 15 and 16 have been rejected under 35 U.S.C. Sec. 103 as allegedly obvious in view of Wong *et al.*, U.S. Patent No. 5,705,194. Applicants respectfully traverse the rejection.

By this amendment, the limitations of claim 2 have been incorporated into claim 1. Claim 2 has not been subjected to the present rejection. Applicants submit that their invention as set forth in claim 1 as amended is unobvious over the cited reference.

Claims 15 and 16 depend upon claim 1. Applicants submit that the more specific dependent claims are also non-obvious.

Therefore, Applicants respectfully request withdrawal of the 35 U.S.C. Sec. 103 rejection over Wong *et al.* '194.

IX. Conclusion

Reconsideration and allowance of claims 1, 3-16, 22, 23 and 29 is requested. Should the Examiner believe that a conference with Applicants' Attorney would advance prosecution of this application, he is respectfully invited to call Applicants' Attorney at the number below.

Respectfully submitted,

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Review

The use of gonadotrophin-releasing hormone (GnRH) agonists in early and advanced breast cancer in pre- and perimenopausal women

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Abstract

Gonadotrophin-releasing hormone (GnRH) agonists, in particular goserelin ('Zoladex'), are increasingly being used for the treatment of breast cancer in women with functioning ovaries. They act by downregulating pituitary GnRH receptors, thereby suppressing the release of luteinising hormone (LH) and follicle stimulating hormone (FSH), which, in turn, reduce the main source of oestradiol production in the ovaries. GnRH agonists have been shown to be as effective therapeutically as surgical ovarian ablation in pre- and perimenopausal women with advanced breast cancer. The combination of a GnRH agonist such as goserelin with the peripheral oestrogen antagonist, tamoxifen, may be used to produce 'combined oestrogen blockade'. In advanced breast cancer, this regimen prolongs progression-free survival and increases both the response rate and duration relative to the use of a GnRH agonist alone. In patients with early breast cancer, the addition of goserelin to 'standard treatment' (i.e. surgery±tamoxifen, chemotherapy or radiotherapy) results in a significant benefit in recurrence-free survival and overall survival. This benefit was most apparent in patients with oestrogen receptor (ER) +ve tumours. Goserelin, when used either alone or in combination with tamoxifen as an adjuvant systemic therapy in women with ER +ve tumours, has been shown in clinical trials to produce recurrence-free survival rates equivalent to cytotoxic chemotherapy such as cyclophosphamide, methotrexate, 5-fluorouracil (CMF). Evidence suggests that at least part of the effect of adjuvant cytotoxic chemotherapy in premenopausal women is produced by ovarian ablation. Endocrine therapy with goserelin or goserelin plus tamoxifen should now be considered a treatment option in the management of premenopausal women with ER +ve early breast cancer.

Keywords: GnRH agonist; Goserelin; Tamoxifen; Adjuvant; Breast cancer; 'Zoladex'; Ovarian suppression; Castration

1. Introduction

More than 100 years ago, Beatson demonstrated that endocrine ablation by oophorectomy was accompanied by a regression of disease in a patient with advanced breast cancer [1]. He also understood that control of lactation in cows that had recently calved was mediated by the ovaries and not through the central nervous system; an early description of hormone action.

In the premenopausal woman, gonadotrophin-releasing hormone (GnRH) is released from the hypothalamus in a pulsatile fashion (pulses approximately every 90 min) under normal physiological conditions and is carried by the portal veins directly to the anterior pituitary gland where it binds to GnRH receptors, stimulating the release of luteinising hormone (LH) and follicle stimulating hormone (FSH) [2]. The occupied receptors form clusters and are taken up into the pituitary cells. These inactivated receptors are replaced by newly synthesised receptors on the cell surface, ready for the next pulse of GnRH. LH stimulates the ovaries to produce oestrogens, including oestradiol.

This process is responsible for producing up to 90% of circulating oestradiol, depending on the phase of the menstrual cycle; the remainder in premenopausal women, and all in postmenopausal women, is produced by aromatisation of androgens by the adrenal glands

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and other tissues. Therapeutic approaches to the endocrine treatment of breast cancer have targeted both this regulatory pathway and the point of peripheral action of oestrogens by oestrogen receptor (ER) blockade.

Following Beatson's pioneering work, ovarian ablation by means of surgical castration, or later by irradiation, became a well-established means of endocrine manipulation in premenopausal women with advanced breast cancer. Response rates in metastatic breast cancer range from approximately 30% in unselected patients [3,4] up to 79% in patients with ER +ve tumours [5,6].

Surgical ablation is a relatively straightforward procedure with little perioperative morbidity, but it is an invasive method and has the potential of psychological upset, particularly in non-responders. An alternative to surgery is to use irradiation. Radiation ablation produces similar response rates to surgical ablation, but also has occasional long-term complications, takes longer to achieve castrate levels of oestradiol and long-term suppression is unreliable. Both procedures are irreversible and since only around one-third of patients may respond to this type of therapy, a sizeable proportion will have undergone these procedures for no clinical benefit.

The possibility of being able to suppress oestradiol levels medically is an attractive option. Use of GnRH agonists to downregulate GnRH production by the hypothalamus effectively produces a 'medical ablation' of the ovaries which is potentially reversible on discontinuation of therapy. Data showing the effectiveness of GnRH agonists in patients with advanced breast cancer were first published using buserelin [7], and goserelin ('Zoladex') [8]. Other members of this class include leuprorelin and triptorelin. Goserelin is the most extensively investigated member of the GnRH agonists, accounting for over 90% of the published data on GnRH agonists in the treatment of breast cancer, and no comparisons between the GnRH agonists have been reported.

During long-term administration, the GnRH receptors are effectively over-stimulated, resulting in down-regulation of the GnRH receptors in the pituitary gland. Experience over the last decade shows that goserelin provides an effective means of 'medical oophorectomy' for as long as the agent is administered. In the clinical setting, goserelin has been widely tested in early and advanced breast cancers. Ovarian suppression with goserelin has a more rapid onset than ovarian irradiation and its effects on ovarian function are reversible in most women [9], which may be important in premenopausal women in the adjuvant setting.

1.1. Pharmacodynamics of GnRH agonist therapy

The pharmacodynamics of goserelin in breast cancer were studied in the 1980s. The early work used daily subcutaneous (s.c.) injections [8], which were superseded by the monthly depot formulation of goserelin (3.6 mg) [8] that is now used in clinical practice. Goserelin produced an initial rise in LH and FSH for 7–10 days followed by a decrease in LH and FSH after 14–21 days in premenopausal women with advanced breast cancer. Progesterone and oestradiol levels followed those of the gonadotrophins with a short-lived rise followed by a fall to levels seen in oophorectomised or postmenopausal women 14–21 days after continuous administration. Similar endocrine responses were seen in women who received either daily (s.c.) injections or the monthly depot formulation of goserelin [8].

The ability of the monthly depot of goserelin to suppress serum concentrations of FSH, LH and oestradiol was also demonstrated in a study of 118 evaluable pre- and perimenopausal patients with metastatic breast cancer. This study showed that mean serum oestradiol values fell into the range seen in castrated or postmenopausal women (i.e. <30 pg/ml) after 2–3 weeks of treatment and suppression was maintained throughout therapy (up to 24 months) [10].

1.2. Effects of GnRH agonists on ovarian histology

While GnRH agonists suppress gonadotrophins and oestrogens to castrate levels in premenopausal women, leading to the cessation of menses, it is important to understand the effects of GnRH agonists on folliculogenesis and follicular maturation since these may indicate the potential for maintaining fertility following discontinuation of treatment.

The expectation was that goserelin would inhibit folliculogenesis by decreasing FSH and LH to very low levels. However, a study of ovarian histology showed that goserelin inhibits follicular maturation, but not folliculogenesis [11]. This is an important finding for young women with breast cancer as it may be expected that after discontinuation of goserelin, and the return of the pretreatment endocrine environment, maturation of the follicles will occur with a resumption of fertility. This would not be possible following surgical oophorectomy, irradiation or chemotherapeutic ablation.

1.3. Pharmacodynamics of GnRH agonists and tamoxifen in combination

An important question is whether there are any advantages to 'combined oestrogen blockade' using goserelin and tamoxifen in premenopausal women, gaining a double effect by first rendering the patient postmenopausal by the use of goserelin and then an additional effect from the use of tamoxifen, as in postmenopausal women.

In addition to the potentially greater antitumour effect, tamoxifen may theoretically shield the tumour

from the initial surge in oestradiol seen with goserelin monotherapy. In addition, goserelin has been shown to prevent the intermittent spikes of oestradiol seen with tamoxifen monotherapy [12]. Co-treatment with a GnRH agonist has been shown to prevent the formation of ovarian cysts when added to tamoxifen therapy [13].

The pharmacodynamics of goserelin in combination with tamoxifen have been compared with the effects of goserelin alone in pre- and perimenopausal women with advanced breast cancer. The initial surge in gonadotrophin levels with goserelin alone or in combination with tamoxifen lasted 7–10 days and was followed by a profound suppression of LH and FSH on both regimens. The combination had a more marked effect on serum FSH than goserelin alone [14] and the combination prevented the upward drift of FSH seen with goserelin alone. Both regimens suppressed serum oestradiol and progesterone to levels equivalent to those seen after surgical oophorectomy and there were no peaks in serum oestradiol with the combination.

2. GnRH agonists in advanced breast cancer

2.1. Clinical aspects of GnRH agonist monotherapy

The effectiveness of goserelin in the treatment of advanced breast cancer in pre- and perimenopausal women was established in a combined analysis of 29 phase II studies [15]. A total of 333 pre- and perimenopausal women with histologically-confirmed stage III or IV breast cancers was recruited between 1982 and 1988. Each patient received a goserelin depot injection every 28 days.

Of the 333 patients recruited, 228 patients were evaluable for efficacy. Treatment with goserelin gave a median survival of 26.5 months (range: 0.8–69 months); survival was longer in ER +ve patients (33.1 months) than in ER −ve patients (15.9 months) [16]. The objective clinical response rate was 36% (83/228 patients) and the subjective response rate was 68% (97/142 patients). The highest response rates were observed in patients with ER +ve tumours (objective response 44%, versus 31% for ER −ve patients). The median duration of response was 44 weeks (4–160 weeks). The reported response rate in ER −ve disease was higher than expected. There is no proven explanation for this result, but it may reflect a less strict attention to detail (for example in the collection of fresh tumour tissue) in the measurement of ER prevalent at that time.

The overall response rates were similar to those expected from surgical ablation in premenopausal women with advanced breast cancer. In a randomised trial of medical (GnRH agonist) versus surgical ovarian ablation, patients with ER and/or progesterone receptor (PgR) +ve tumours were assigned to receive goserelin depot ($n=69$) or surgical oophorectomy ($n=67$) [17]. The two treatment modalities were comparable in terms of objective clinical response (goserelin 31%; oophorectomy 27%) and stable disease (goserelin 28%; oophorectomy 26%). Overall and progression-free survival were similar for both goserelin and oophorectomy.

The endocrine and clinical data support the use of goserelin as a means of providing 'medical oophorectomy' in pre- and perimenopausal women with advanced breast cancer. Goserelin is well tolerated [15] and offers a non-invasive and reversible alternative to surgical ablation.

Data from other GnRH agonists have also demonstrated the efficacy of GnRH agonist monotherapy in premenopausal patients with advanced breast cancer. In several separate studies of leuprorelin, objective response rates of between 34 and 44% were reported [18–21]. Studies with buserelin monotherapy have reported objective response rates of 14–41% [22–25], while treatment with triptorelin has been shown to produce objective response rates of between 30 and 70% in premenopausal patients with advanced breast cancer [26–28].

2.2. Combination of GnRH agonists and tamoxifen in advanced breast cancer

A pilot study showed an overall response rate of 25% [29] with the combination of goserelin and tamoxifen in premenopausal women with advanced breast cancer.

Four randomised trials ([24,30,31] T. Tominaga, unpublished) have been undertaken to address whether combination therapy with a GnRH agonist and tamoxifen is superior to treatment with a GnRH agonist alone in pre- and perimenopausal patients with advanced breast cancer. Three of the studies used goserelin (79% of patients) and one used buserelin.

The study treatments compared initial combination therapy versus initial GnRH agonist alone: progression was assessed as the first progression after initiation of either therapy. In one of the studies, the largest of the four (ICI 2302) [31], the study was also designed to compare initial combination therapy with a sequential policy, where tamoxifen was added to goserelin when signs of progression were observed on goserelin monotherapy.

In the first of these trials, 85 perimenopausal patients with ER +ve metastatic breast cancer received ovarian ablation (surgery or irradiation), ovarian ablation plus tamoxifen, goserelin or goserelin plus tamoxifen. Objective response rates were 46% with ovarian ablation, 11% with ovarian ablation plus tamoxifen, 27% with goserelin and 45% with goserelin plus tamoxifen; differences between the treatments were not significant. There was no significant difference in the time to treatment progression (TTP) or time to death (TTD)

between the groups. The authors concluded that goserelin was of comparable efficacy to ovarian ablation by surgery or irradiation and that tamoxifen enhanced the efficacy of goserelin, but not ovarian ablation [30].

In a second study of 161 premenopausal patients with advanced breast cancer, combined treatment with buserelin plus tamoxifen was significantly superior to either treatment alone for both TTP and TTD. Time to progression was 9.7, 6.3 and 5.6 months for combined, buserelin and tamoxifen groups, respectively. There was no significant difference in the objectives response rates between treatments (48, 34 and 28%, for combined, buserelin and tamoxifen groups, respectively), but clinical benefit rates (including patients in whom their disease did not progress for > 6 months) were statistically greater for the combined treatment group (75%) compared with buserelin or tamoxifen alone (62 and 44%, respectively; $P=0.007$) [24].

The third study compared the efficacy of goserelin alone and in combination with tamoxifen in 318 pre/perimenopausal patients with advanced breast cancer. In this study, objective response rates were comparable between treatments (31 and 38% in goserelin and goserelin plus tamoxifen groups, respectively). Although TTP was significantly greater with the combination treatment (23 weeks versus 28 weeks for goserelin alone; $P=0.03$), there was no significant difference between the treatments for survival [31].

These trials have been combined in a meta-analysis [32]. At the time of analysis, approximately 70% of the patients had died. The meta-analysis reported that initial combination treatment led to a 22% lower risk of mortality ($P=0.02$), and a 30% lower risk of disease progression ($P<0.001$), than was seen with GnRH agonist monotherapy (Table 1). The objective clinical response rate was also significantly higher with combination therapy (39%) than with initial monotherapy (30%). The duration of response was almost twice as long with combination therapy (median: 19.4 months) as with GnRH agonist monotherapy (median: 11.3 months).

Although the side-effects of the combination were numerically greater in the ICI 2302 trial, this trial concluded that there were no additional safety issues associated without providing substantive data with the combination therapy [31].

The ICI 2302 trial (included in the meta-analysis) was the only trial which had intended to compare the effect of initiating treatment with the combination of goserelin plus tamoxifen versus the sequence of adding tamoxifen to goserelin on disease progression. In 50% of progressions this addition of tamoxifen did not occur because patients had rapidly progressing disease and alternative therapy was deemed appropriate [31]. Nevertheless, in the 71 patients who received subsequent treatment with tamoxifen after progression on goserelin, 18% had an objective response and 41% had stable disease, giving a total of 59% with clinical benefit. The median time to further progression in this patient group was 20 weeks. These data suggest that there may be no benefit of combined over sequential endocrine therapy as long as the second agent is given at recurrence.

In clinical practice, if patients have rapidly progressing disease, combined therapy gives the chance of an improved response and may offer psychological and quality-of-life advantages. An advantage to using the combination in all patients is that they have an unbroken disease-free period whereas with the sequence, although the overall length of response may be the same or longer, the patient has disease progression during this time.

However, combined therapy does use two treatment modalities simultaneously and may restrict the options available for second- or third-line treatments. Therefore, patients with relatively indolent disease, such as ER +ve bone metastases, may be considered for sequential therapy, since the clinician can expect, with some confidence, to have the opportunity to add tamoxifen sequentially on disease progression.

2.3. Combination of GnRH agonists and aromatase inhibitors in advanced breast cancer

The use of GnRH agonists in premenopausal women essentially renders them postmenopausal. This allows the use of agents, such as aromatase inhibitors (AIs), that are traditionally reserved for the treatment of

Table 1
Summary of meta-analysis results comparing GnRH agonist therapy with GnRH agonist + tamoxifen in women with advanced breast cancer

Endpoint	GnRH-A alone ($n=256$)	GnRH-A + TAM ($n=250$)	Hazard ratio/odds ratio (95% CI)	P value
Primary				
Median survival (years)	2.5	2.9	0.78 (95% CI 0.63–0.96)	0.02
Secondary				
Median progression-free survival (months)	5.4	8.7	0.70 (95% CI 0.58–0.85)	<0.001
Objective response (%)	29.7	38.8	0.67 (95% CI 0.46–0.96)	0.03
Number (%) responders	76 (30%)	97 (39%)	–	–
Median duration of response (months)	11.3	19.4	–	–

GnRH-A, GnRH agonist; TAM, tamoxifen; GnRH, gonadotrophin-releasing hormone; 95% CI, 95% Confidence Interval.

breast cancer in postmenopausal patients. Of note, combination treatment with goserelin and either vorozole or formestane has been shown to produce a more profound suppression of oestrogen levels than treatment with goserelin alone [33,34]. Similarly, in 16 premenopausal women with metastatic or locally advanced breast cancer previously treated with goserelin plus tamoxifen, substituting anastrozole for tamoxifen upon disease progression produced a 76% further reduction in the serum oestradiol level ($P < 0.05$) above that initially achieved with goserelin plus tamoxifen (89% reduction from pre-treatment levels; $P < 0.05$). Clinically, the combination of goserelin plus anastrozole resulted in clinical benefit in 12 (75%) patients, with a median duration of remission of > 17 months [35]. The combination of goserelin plus formestane has also been shown to produce responses in patients progressing after an initial response to goserelin alone [34]. These data suggest that using GnRH agonists to convert premenopausal patients to a postmenopausal state will increase the range of endocrine treatments available for premenopausal advanced breast cancer patients. In particular, the combination of GnRH agonists with AIs provides a valuable treatment option for those patients who have progressed on tamoxifen therapy.

3. GnRH agonists as adjuvant therapy for early breast cancer

The primary management of early breast cancer is surgical removal of the tumour by mastectomy or lumpectomy, ± radiotherapy. The choice of adjuvant systemic therapy for early breast cancer depends on the patient's prognosis, menopausal status and ER status. Tamoxifen is the established adjuvant treatment for postmenopausal women with hormone-sensitive early breast cancer. For premenopausal patients with hormone-sensitive disease, treatment options include chemotherapy, tamoxifen, ovarian ablation and combinations of these therapies.

The value of adjuvant ovarian ablation (by surgical oophorectomy or ovarian irradiation) in premenopausal women was clearly established by the Early Breast Cancer Clinical Trials Group (EBCCTG) in 1996 [36]. The overview demonstrated a highly significant improvement for these measures over controls in both recurrence-free survival and overall survival in women < 50 years of age. The magnitude of the effect of ovarian suppression demonstrated in patients unselected by ER status is similar to that produced by postoperative chemotherapy: benefit was seen irrespective of the nodal status at diagnosis. The response to ovarian suppression or oophorectomy is greater among patients who are ER +ve. Therefore, the results in patients unselected by ER status are likely to be an underestimate of the benefit of ovarian ablation in patients with ER +ve tumours.

Recently, the results of five comparative trials of adjuvant hormonal therapy using GnRH agonists alone [37,38] or in combination with tamoxifen [39–41], versus cytotoxic chemotherapy have shown at least equivalence of effect in premenopausal women with ER +ve tumours (see below).

3.1. Efficacy of GnRH agonists in adjuvant therapy

The Zoladex Early Breast Cancer Research Association (ZEBRA) trial compared six cycles of CMF (cyclophosphamide, methotrexate, 5-fluorouracil) with 2 years of treatment with goserelin in 1640 pre- and perimenopausal patients aged ≤50 years with node +ve disease [37]. In the patients with ER +ve tumours (74%), goserelin was equivalent to CMF in terms of disease-free survival (DFS) at a median follow-up of 6 years (hazard ratio (HR) = 1.01; 95% confidence interval (CI) 0.84–1.20). In patients with ER −ve tumours, goserelin was inferior to CMF for DFS (HR = 1.76; 95% CI 1.27–2.44) (Fig. 1).

The Italian Breast Cancer Adjuvant Study Group (GROCTA) 02 trial ($n = 244$) [40,42] evaluated adjuvant treatment with CMF versus ovarian suppression (oophorectomy $n = 6$, irradiation $n = 31$, goserelin $n = 87$) plus tamoxifen in patients with ER +ve breast cancer. This trial showed that at a median follow-up of over 7 years, there were no differences with respect to either DFS or overall survival in patients treated with ovarian suppression plus tamoxifen compared with those treated with CMF. For tamoxifen plus ovarian suppression compared with CMF, the HR of relapse was 0.95 (95% CI 0.62–1.46; $P = 0.8$) and of death was 0.71 (95% CI 0.38–1.31; $P = 0.3$).

The Austrian Breast and Colorectal Cancer Study Group (ABCSG) trial ($n = 1088$) [39] in women with ER +ve and/or PgR +ve tumours showed the combination of goserelin and tamoxifen to be more effective than CMF chemotherapy in terms of recurrence-free survival ($P < 0.02$) over a median follow-up of 50 months. Overall survival data are currently immature and show no statistical difference between the two treatment groups.

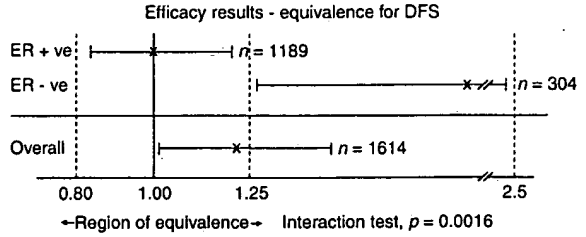

Fig. 1. Disease-free survival (DFS) by Oestrogen Receptor (ER) status in the Zoladex Early Breast Cancer Research Association (ZEBRA) trial.

A French study [41] compared triptorelin plus tamoxifen for 3 years with FEC (5-fluorouracil, epirubicin and cyclophosphamide) for six cycles in 333 premenopausal women with hormone receptor +ve disease. Overall survival and DFS over 54 months were similar in both arms ($P=0.18$, 0.12, respectively).

The Takeda Adjuvant Breast Cancer Study with Leuprorelin (Enantone) (TABLE) study [38] compared six cycles of CMF with 2 years of leuprorelin in 600 pre/perimenopausal patients: over 90% of patients in each group were hormone receptor +ve. Progression-free survival rates at 2 years were not significantly different between the two treatment arms (67.7% with hormone treatment and 63.1% with CMF).

Three other trials did not directly compare cytotoxic therapy with hormonal therapy, but are of interest.

The Zoladex In Premenopausal Patients (ZIPP) trial [43] determined the effect of adding goserelin to standard adjuvant treatment (surgery±radiotherapy ±chemotherapy±tamoxifen) compared with the effect of standard treatment alone in women <50 years of age. At a median follow-up of 66 months, the event-free survival was significantly longer for patients who received goserelin in addition to standard therapy compared with those who did not (HR=0.80; 95% CI 0.70–0.92; $P<0.001$). Overall survival was also significantly prolonged (HR=0.82; 95% CI: 0.67–0.99; $P=0.04$). Subgroup analysis suggested that goserelin had its greatest effect in patients with ER +ve tumours who did not receive chemotherapy, but none of the tests for interaction were significant [44].

A study by the Eastern Cooperative Oncology Group (ECOG)/South West Oncology Group (SWOG)/CALGB (Cancer and Leukaemia Group B) [45] in ER +ve patients compared the addition of goserelin with or without tamoxifen to CAF (cyclophosphamide, doxorubicin and 5-fluorouracil). At a median follow-up of 6.2 years, this study (INT-0101) showed a trend in favour of improved DFS for the addition of goserelin to CAF, although this just failed to reach statistical significance. The addition of goserelin plus tamoxifen to CAF chemotherapy resulted in a significant benefit in DFS over the use of CAF plus goserelin ($P<0.01$). The percentages of patients disease-free after 5 years for CAF alone, CAF plus goserelin and CAF plus goserelin and tamoxifen were 67, 70 and 77%, respectively. In this study, the addition of tamoxifen seemed to be more efficacious in those women with postmenopausal oestradiol (E_2) levels at the end of adjuvant CAF therapy, while goserelin was more beneficial in women with premenopausal oestradiol levels at the end of CAF therapy (data not shown). This trial looked at the addition of goserelin to cytotoxic chemotherapy, but did not evaluate the more interesting question of the advantage of the addition of cytotoxic therapy to goserelin therapy. This was addressed in the International Breast Cancer Study Group (IBCSG) Trial VIII.

The IBCSG Trial VIII was initially designed to compare six cycles of CMF with either 2 years goserelin, six cycles of CMF followed by 18 months goserelin or no treatment in premenopausal women with node −ve early breast cancer. Enrolment into the untreated arm was discontinued when other trials showed that adjuvant therapy improves survival in node −ve disease. Interim analysis of 200 patients from the four original arms confirmed the benefits of adjuvant therapy in this patient group; 5-year DFS of treated patients was significantly longer than for untreated controls (77% versus 60%; $P=0.02$) [46]. Recent data from this study show that in patients with ER +ve tumours, there was no significant difference between the treatment groups for 5-year DFS (goserelin 81%; CMF 81%; CMF plus goserelin 88%). Thus, the authors conclude that the value of adding chemotherapy to goserelin is questionable in patients with node −ve, hormone-sensitive disease [47].

In a small study ($n=92$) in premenopausal women with ER +ve disease, the addition of goserelin to epirubicin provided no statistically significant benefit in terms of overall survival or DFS in addition to that seen with chemotherapy alone [48].

3.2. Tolerability of GnRH agonists in adjuvant therapy

It is well established that chemotherapy is associated with toxicities such as: myelosuppression, gastrointestinal effects (anorexia, nausea, vomiting and diarrhoea), mucositis, stomatitis, alopecia and general fatigue. Treatment with GnRH agonists is associated with menopausal side-effects such as hot flushes and vaginal dryness. There are few data available directly comparing the tolerability profiles of these treatment modalities.

In the ZEBRA study, CMF was associated with more nausea/vomiting, alopecia and infection than goserelin, while goserelin initially produced more side-effects related to oestrogen suppression. Once adjuvant therapy had ceased, the incidence of menopausal side-effects was greater in women given cytotoxic chemotherapy than in those treated with goserelin. The increased incidence of menopausal side-effects in the chemotherapy group is associated with the high level of permanent amenorrhoea in this group (at 3 years 79% of patients treated with CMF were amenorrhoeic) [9].

Of note, overall quality of life was significantly better with goserelin in the first 3–6 months of the trial ($P<0.0001$) and did not differ significantly between treatments thereafter.

These results are supported by the TABLE study [38] in which serious adverse events were reported by 3.6% of those receiving hormonal therapy and 15.4% of those receiving cytotoxic chemotherapy.

In summary, these trials have shown that hormonal therapy with goserelin (±tamoxifen) is at least as effective as cytotoxic regimens in premenopausal women with ER +ve tumours, and is not associated with the distressing side-effects of chemotherapy. In addition, goserelin±tamoxifen can provide benefit when added to standard adjuvant therapy.

3.3. The role of amenorrhoea in women with ER +ve tumours treated with cytotoxic agents

Cytotoxic regimens render 20–80% of premenopausal women amenorrhoeic, depending on their age, the cytotoxic agent and cumulative dose used [9,49,50]. Since castration by any means has a powerful risk-reducing effect in the adjuvant setting [36], it follows that some of the effect seen with adjuvant chemotherapy is brought about by the side-effect of chemical castration.

Several studies have shown that amenorrhoea is a significant prognostic factor in premenopausal women with early breast cancer treated with chemotherapy: amenorrhoeic patients showed improved disease-free survival compared with those women who continued to menstruate [50–52]. In the Austrian study [50], 80% of patients receiving cytotoxic chemotherapy became amenorrhoeic and this subgroup of patients had significantly fewer recurrences and improved overall survival compared with the 20% who had continued menses through and after chemotherapy. In the ZEBRA trial, recurrence-free survival was superior in those treated with CMF who became amenorrhoeic compared with those who did not [37].

3.4. Prescription of adjuvant therapy for premenopausal ER +ve tumours

Adjuvant treatment should be tailored to need, i.e. prognosis. There are alternative strategies for classifying patients into low, moderate and high risk (such as the Nottingham Prognostic Index [53], which we favour). A group of patients with a good prognosis can be identified, in whom the potential absolute benefit of adjuvant therapy is very small and adjuvant therapy is not indicated.

In those patients with ER +ve tumours and moderate prognoses, the authors believe that the risk–benefit analysis favours adjuvant hormone therapy alone, although clearly this should be discussed with each individual patient. In the remaining patients with poor prognoses, a considerable absolute gain can be expected from hormonal therapy, but given the high percentage of recurrences, there is probably a further absolute gain from the application of cytotoxic chemotherapy and the authors would advise chemo-endocrine therapy in these patients.

This tailored approach highlights the difference between basing treatment on relative risk for all versus absolute risk for an individual patient.

4. Conclusions

The use of GnRH agonists such as goserelin is as effective as surgical oophorectomy or radiotherapeutic ovarian ablation in the management of premenopausal women with ER +ve advanced breast cancer and provides a non-invasive, reversible method of ovarian suppression. In early breast cancer, GnRH agonists have been shown to be as effective as CMF chemotherapy in hormone-sensitive disease.

The combination of a GnRH agonist with tamoxifen as first-line therapy for advanced disease can be used to improve the initial response rate and considerably lengthen the duration of response. In early disease, goserelin plus tamoxifen has been shown to be more effective that CMF chemotherapy.

Current treatment guidelines from St Gallen and the European Society of Mastology (EUSOMA) now recommend the use of a GnRH agonist±tamoxifen as treatment for premenopausal women with hormone-sensitive early breast cancer.

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Gonadotrophin-Releasing Hormone Agonists

A Guide to Use and Selection

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Contents

Summary

The development of superactive analogues of gonadotrophin-releasing hormone (GnRH) represents one of the most important new pharmaceutical contributions of the last 2 decades. This class of drugs is now available worldwide and is successfully employed in the management of precocious puberty, ovulation induction, prostatic cancer, premenopausal breast cancer, endometriosis, uterine leiomyoma, and for the preparation of female patients undergoing laparotomic,

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vaginal or endoscopic surgery. GnRH agonists are also applied with some success in other clinical conditions such as catamenial disorders, hyperandrogenism and menometrorrhagia. Studies are under way to identify other potential clinical applications such as other forms of cancer.

1. Pharmacology of Gonadotrophin-Releasing Hormone Agonists

1.1 Structure-Related Characteristics

Over 20 years have now passed since the pioneering studies that allowed basic strategies for the preparation of superactive gonadotrophin-releasing hormone (GnRH) agonists. The 2 fundamental modifications that permitted synthesis of these compounds are the replacement of the tenth amino acid (glycine) of the native GnRH sequence with an ethylamide (NEt) residue[1] and the substitution of the sixth amino acid (glycine) with other more lipophilic D-amino acids such as D-Phe, D-Leu or D-Trp.[2] Other structural modifications that permit enhanced potency and that were incorporated in the design of clinically available GnRH agonists include the use of more complex amino acid molecules in position 6 [D-Ser (tBu), D-His (Bzl), D-Nal(2)][3] and/or in position 10 [aza-Gly],[4] and the N-Me-Leu modification in position 7[5] (table I).

Most of these modifications result in more hydrophobic compounds that are more stable than the native GnRH molecule because of greater conformational stability of a β-II type bend in the analogue molecule. Receptor affinity and *in vitro* potency appear to be directly proportional to the hydrophobicity of each GnRH agonist.[6] In addition, the more hydrophobic GnRH agonists are more resistant to enzyme degradation and bind more strongly to plasma proteins and body tissues, thus decreasing renal excretion and prolonging drug half-life.[6]

However, as greater receptor affinity, longer half-life and decreased degradation are all enhanced by increased hydrophobicity and act synergistically to increase GnRH agonist potency, the identification of the specific role of each of these factors is difficult. Furthermore, enhanced hydrophobicity may not be the only factor contributing to agonist potency.[7] For example, when the more hydrophobic position 6 amino acid substitutions are employed, additional modifications such as Pro^9-NEt do not appear to further enhance GnRH agonist potency.[8] Thus, while [$D\text{-}Leu^6$, Pro^9-NEt]GnRH (leuprorelin) is more potent than [$D\text{-}Leu^6$]GnRH, [$D\text{-}Trp^6$, Pro^9-NEt]GnRH (deslorelin) and [$D\text{-}Trp^6$]GnRH (triptorelin) appear to be roughly equipotent.[8]

Some differences in potency estimates may also be related to the reduced relevance in primates of the post-proline cleaving enzyme that catalyses the separation of the Pro^9-Gly^{10} bond.[9] Finally, inconsistencies in potency estimations between *in vitro* and *in vivo* assays, and between rodent and primate models are common and do not allow assessment of potency unless these studies are performed under the same assay and experimental conditions.

1.2 Mechanisms of Action

GnRH agonists all exert their action through the suppression of endogenous gonadotrophins. A hypogonadal condition ensues, with profound reductions in gonadal steroid secretion and gamete maturation. Therapeutically proven actions of GnRH agonists are exclusively related to the virtually complete elimination of gonadotrophin and/or gonadal steroid stimulatory effects on the reproductive system or on pathological tissues.

At the pituitary level, GnRH agonists affect GnRH receptors and the postreceptor message that provides the normal input for gonadotrophin synthesis and secretion.[10] This long term inhibitory action is preceded by a transient (1 to 2 weeks) stimulatory phase associated with elevated gonadotrophin and gonadal steroid concentrations. This flare-up phase is usually clinically irrelevant. However, in specific conditions the flare-up phe-

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nomenon can cause dangerous complications (as in prostatic cancer) or has been exploited to enhance therapeutic efficacy (as in ovulation induction).

Physiological and/or therapeutic mechanisms of action of GnRH agonists unrelated to pituitary desensitisation have been suggested, as outlined in section 4. Unfortunately, when these hypotheses were clinically tested, no efficacy unrelated to the classical GnRH agonist-induced secondary hypogonadism could be demonstrated.

An interesting application related to the identification of GnRH receptors on tumour tissue is the coupling of GnRH agonists or antagonists with cytotoxic radicals,[11] which would permit the achievement of higher concentrations and activity of cytotoxic compounds at the tumour level. Additional studies will, however, be required to demonstrate the clinical efficacy of this approach.

2. Route of Administration and Delivery Systems

All GnRH agonists now clinically available are highly potent and capable of inducing profound pituitary and gonadal suppression when given in proper amounts. Thus, all the agonists reported in table I can be employed for the indications listed in this article. Nevertheless, different administration routes affect drug absorption and may cause incomplete pituitary suppression. Different GnRH agonists have been formulated to be administered subcutaneously, intranasally, or as intramuscular or subcutaneous long-acting depot injections, as noted in table I.

When choosing a GnRH agonist dosage it should be remembered that complications or adverse effects from overdosage of these drugs have never been reported. Conversely, inadequate pituitary/gonadal suppression may prevent full therapeutic efficacy of these compounds. Thus, when employing short-acting subcutaneous preparations, a daily GnRH agonist dosage in excess of that suggested by the manufacturer can be employed without concern if insufficient suppression is present or suspected. Partial pituitary suppression with borderline GnRH agonist dosages has been suggested to reduce adverse effects such as bone loss. However, as it is virtually impossible to identify the precise GnRH agonist dose required for minimum suppression in each patient, this approach may result in a loss of therapeutic efficacy and should be avoided.

Dosage adjustments are more difficult with intranasal or depot formulations. Only about 3% of the GnRH agonist administered intranasally is absorbed and inadequate therapeutic efficacy has been reported with this route.[12] Nafarelin is one of the most hydrophobic and, thus, potent GnRH agonists currently available and appears to be more suitable for intranasal administration. Nevertheless, it is unlikely that even nafarelin can provide the same depth of pituitary suppression offered by other GnRH agonists delivered subcutaneously at high doses.

Table I. Name, amino acid substitutions and routes of administration of available gonadotrophin-releasing hormone agonists

Name	Substitutions			Routes
	position 6	position 7	position 10	
Leuprorelin	D-Leu		NEt	SC, depot (IM or SC)
Triptorelin	D-Trp			SC, depot (IM or SC)
Deslorelin	D-Trp		NEt	SC
Lutrelin	D-Trp	N-Me-Leu	NEt	SC
Buserelin	D-Ser(tBu)		NEt	IN, SC, depot (SC)
Goserelin	D-Ser(tBu)		aza-Gly	Depot (SC)
Histrelin	D-His(Bzl)		NEt	SC
Nafarelin	D-Nal(2)			IN
Meterelin	D-Me-Trp(2)		NEt	Depot

Abbreviations: IM = intramuscular; IN = intranasal; SC = subcutaneous.

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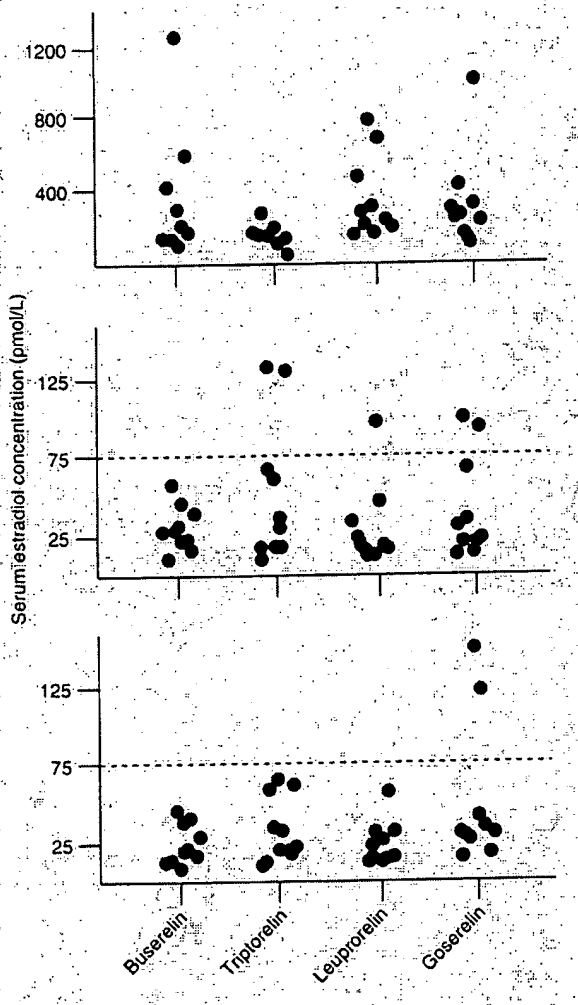

Fig. 1. Serum estradiol concentrations before (**top**), at the beginning of the third month (**middle**), and at the end of the third month (**bottom**) of gonadotrophin-releasing hormone (GnRH) suppression with different GnRH agonists. Buserelin was given as a control with a high dose, short-acting subcutaneous regimen (300μg twice a day), while triptorelin (3.6mg), leuprorelin (3.6mg) and goserelin (3.75mg) were administered as depot preparations at monthly intervals. Unsuppressed estrogen concentrations at the end of the third month of GnRH agonist administration were only found in 2 goserelin recipients. The dotted lines indicate the 'castration threshold' of 75 pmol/L (20 pg/ml), the estrogen levels indicative of a lack of ovarian activity (reproduced from reference [16] with permission of the Endocrine Society).

Several GnRH agonists are now available in slow release forms (depot) that permit uninterrupted drug delivery for at least 4 weeks (table I). New delivery systems permitting more prolonged drug administration (2 to 3 months) after a single injection[13] will soon be available. All GnRH agonists presently available are bound to *d,l*-lactide, glycolide copolymers in the shape of microcapsules (leuprorelin, triptorelin) or rods (goserelin). The analogue-carrier complex is injected intramuscularly or subcutaneously. Relatively stable analogue concentrations are achieved thereafter, although differences have been reported for the presently available systems.[14,15]

Adequate pituitary desensitisation can be achieved in most treatment cycles with intramuscular microcapsule depot preparation.[16] However, incomplete suppression of gonadal steroids was reported with goserelin depot, which must be administered subcutaneously[16,17] (fig. 1). This phenomenon may be related to different diffusion dynamics of rod-shaped depot agonists[14] or the subcutaneous versus intramuscular mode of administration. This issue is particularly relevant considering that day-to-day dosage adjustments are not possible with depot formulations and that complete gonadal steroid suppression can be critical to some clinical applications of GnRH agonists, such as cancer.

3. Adverse Effects

All the adverse effects of GnRH agonists are dependent on the profound hyposecretion of gonadal steroids induced by these compounds. No adverse effects derive from a direct action of GnRH agonists. Other mechanisms responsible for GnRH agonist adverse effects have been suggested, but never demonstrated. In postpubertal patients, vasomotor symptoms (hot flashes) are virtually inescapable. In male patients impotence is present during GnRH agonist administration, thus limiting the use of these medications to the area of prostate cancer. Decreased libido also is often reported in women, but is less frequent and less severe than in men. Other common subjective adverse effects include insomnia, mood lability, headaches and vaginal dryness.[18]

Nevertheless, the most clinically significant adverse effect of long term administration of these drugs is bone loss. In a 3-month period, a bone loss of around 3% can be expected.[19] Steroid replacement therapy with estrogen/progestogen combination regimens or progestins alone was tested in several applications and is effective in preventing bone loss and other adverse effects.[19-21] Unfortunately, loss of therapeutic efficacy of GnRH agonists can occur when steroids are co-administered.

4. Clinical Applications

Numerous applications of GnRH agonists were tested since these compounds became clinically available. Although some applications such as male and female contraception[22] did not fulfil early promise, in others, such as precocious puberty and ovulation induction for assisted reproduction, GnRH agonists have become central for treatment success. Table II reports clinical applications for which GnRH agonists have been proven effective; additional applications are discussed in this article. This review updates a previous work published in 1988.[22] Thus, early clinical work in the area of GnRH agonists will not be discussed in the present article.

4.1 Precocious Puberty

Precocious puberty represents one of the earliest and most successful therapeutic applications of GnRH agonists. Several large and well documented studies have shown that GnRH agonist administration in children with precocious puberty blocks the symptomatology of this disorder and reduces growth velocity.[22] These therapeutic effects of GnRH agonists are dependent on the blockade of pituitary gonadotrophins from which derives suspension of gonadal steroidogenesis.

Male and female patients with gonadotrophin-independent precocious puberty (GIP) do not respond to GnRH agonist treatment even after spontaneous puberty.[23] In addition, nocturnal growth hormone (GH) and insulin-like growth factor 1 (IGF-1) could be reduced in children treated with GnRH agonists, thus suggesting sex steroid augmentation of GH secretion.[24,25] However, the finding of diminished GH secretion during GnRH agonist treatment was not confirmed by Sklar et al.[26] who postulated a direct effect of gonadal steroids on bone. Finally, melatonin secretion is not affected by GnRH agonist administration.[27]

The optimal regimen of GnRH agonists to be used in precocious puberty patients is still the subjects of some controversy. Peskovitz et al.[28] tested the effect of subcutaneous deslorelin 1 to 4 μg/kg/day in 29 patients and found that the larger doses (2 to 4 μg/kg/day) appeared to achieve better suppression and, thus, were considered preferable. Parker et al.[29] employed depot leuprorelin and determined that a monthly dose of 7.5mg may be necessary for full therapeutic efficacy. Finally, Laue et al.[30] studied 8 male patients and found that the antiandrogens spironolactone and testolactone can be inadequate for blocking puberty progression, and that a GnRH agonist should be added.

A critical parameter to assess treatment efficacy in precocious puberty is the final stature achieved by patients, since blunted adult height can be expected in most untreated patients. Several recent studies have addressed this issue.[31-33] Long term GnRH agonist administration permits significant improvement in predicted adult height, but the full genetic height potential may not be achieved. However, early initiation of treatment may further increase adult height.[33] The addition of exoge-

Table II. Clinically effective applications of gonadotrophin-releasing hormone agonists

Temporary modification of basic pathogenetic mechanisms
• Precocious puberty
• Ovulation induction
Palliative management of benign disorders (short term treatment)
• Uterine leiomyoma
• Endometriosis
Palliative management of cancer (long term treatment)
• Prostate cancer
• Premenopausal breast cancer
Other applications
• Catamenial disorders
• Hyperandrogenism
• Menometrorrhagia

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nous GH to GnRH agonists in GH-deficient patients with precocious puberty improves predicted height, but not beyond the level achieved in other patients with precocious puberty and normal GH secretion.[34] Thus, GH supplementation may not further improve results obtained with GnRH agonists alone. Finally, the GnRH agonist deslorelin (4 μg/kg/day subcutaneously for 4 years) was used in short children with normally timed puberty and was found to be effective in improving predicted adult height.[35]

Resumption of normal menstrual cyclicity when long term GnRH agonist administration is discontinued is another highly relevant parameter to assess treatment success in precocious puberty. Up to 7 years after patients discontinued therapy, Jay et al.[36] examined 46 female patients treated for at least 2 years and found the timing of menstrual and ovulatory patterns superimposable upon those of normal adolescence. Regular 25- to 35-day cycles were present in 41 and 65% of patients after 1 and 3 or more years from stopping treatment, respectively. Within 2 years, 90% of cycles were ovulatory and 5 pregnancies were recorded in these patients, thus confirming normal reproductive potential.

The experience of the last 14 years, thus, indicates that GnRH agonists are effective and well tolerated and should be considered the optimal treatment option in gonadotrophin-dependent precocious puberty. Bone loss has not been reported in children receiving these agents.

4.2 Ovulation Induction

The concomitant use of GnRH agonists and ovulation induction drugs is a novel, effective and now widely used application of these compounds. GnRH agonists are presently employed in most assisted reproduction programmes worldwide and numerous scientific publications related to this area of investigation have been produced in recent years. GnRH agonists can be combined with pulsatile gonadotrophin-releasing hormone or with exogenous gonadotrophins [both human menopausal gonadotrophins (hMG) and purified follicle-stimulating hormone (FSH)].

When administered with pulsatile GnRH, GnRH agonists are given 4 to 6 weeks before ovulation induction and pulsatile GnRH is started within 12 hours from the last GnRH agonist injection.[37] Only short-acting subcutaneous GnRH agonists and not depot GnRH agonists should be used. We demonstrated that the combined use of GnRH agonists and pulsatile GnRH can improve ovulatory and pregnancy rates in patients with the polycystic ovary syndrome (PCOS) treated with pulsatile GnRH.[38-41] In addition, GnRH agonist pretreatment can markedly reduce the occurrence of multiple pregnancy in patients with PCOS and other disorders, attaining the level encountered in normal women not needing artificial stimulation of ovulation.[41]

Conversely, several different regimens exist for the use of GnRH agonists combined with exogenous gonadotrophins. In the so-called 'long' protocols, GnRH agonists are started in the mid-luteal phase of the menstrual cycle (or earlier), preceding ovulation induction, and are maintained until human chorionic gonadotrophin (hCG) is given (unless a depot GnRH agonist is employed). Thus, pituitary desensitisation and low gonadotrophin and gonadal steroid concentrations are achieved by the time exogenous gonadotrophin administration is begun.

In so-called 'flare-up' (or 'short') protocols, GnRH agonist administration is begun concomitantly with exogenous gonadotrophins and continued until hCG administration. This results in elevated endogenous gonadotrophin concentrations during ovulation induction.

Finally, in 'ultrashort' regimens, GnRH agonists are also started in the early follicular phase of the ovulation induction cycle, but GnRH agonist administration only lasts 3 days.

The mechanisms of therapeutic action of GnRH agonists when combined with exogenous gonadotrophins are complex, controversial, and still not completely understood. In addition to practical considerations regarding the greater ease of sched-

uling GnRH agonist-suppressed patients for ovulation induction and assisted reproduction procedures, the most relevant endocrine effect of GnRH agonists is the abolishment of the endogenous preovulatory luteinising hormone (LH) surge.[42] In the pre-agonist era, the untimely LH surge was associated with premature ovulation and/or follicular luteinisation and, thus, treatment cancellation occurred in a large number of cycles (about 30%).

An additional advantage of the combined use of GnRH agonists and exogenous gonadotrophins is the greater follicle yield that can be achieved in long protocols.[43,44] One possible explanation of this effect is a reduction of intraovarian androgens. GnRH agonists profoundly suppress LH concentrations and consequent gonadal steroidogenetic activity. Intraovarian androgens enhance follicular atresia.[45] Monkey ovaries in flare-up GnRH agonist cycles (a regimen that is characterised by increased serum androgen concentrations)[43] show greater follicular atresia and fewer dissociated granulosa layer follicles.[46] Conversely, long GnRH agonist protocols, both when associated with pulsatile GnRH[38] and with gonadotrophins,[43,47] show lower follicular phase serum androgen concentrations. Thus, lower intraovarian follicular phase concentrations could reduce follicular atresia and promote the development of a greater number of ovarian follicles.

In addition to lowering cycle cancellation rates[48,49] and increasing follicle number in long protocols,[43,49] GnRH agonists may be effective in improving the number and viability of embryos,[49,50] and pregnancy rates.[51-54] However, the issue of whether GnRH agonists actually improve assisted reproduction success rates is still controversial.[48,53,55] Some patients with inadequate response to ovulation induction may additionally profit from GnRH agonist supplementation.[56,57] However, response to gonadotrophins in PCOS does not seem to be improved,[48,58] and patients with premature ovarian failure remain unresponsive.[59]

As previously indicated, long GnRH agonist protocols appear to be superior to flare-up protocols,[44,60,61] and are more widely used in assisted reproduction centres. However, good clinical results have also been reported with the flare-up regimen[62-64] and it should be remembered that long regimens generally require a more prolonged stimulation and the administration of greater dosages of exogenous gonadotrophins. Long regimens, thus, tend to be more expensive, as more GnRH agonist and gonadotrophin are administered. Ultrashort protocols have also been found to be effective,[65,66] but the endogenous LH surge may not always be preventable with these regimens.[64]

Finally, GnRH agonists have been used to replace hCG to provide an endogenous preovulatory LH surge during ovulation induction.[67] This approach may reduce the occurrence of ovarian hyperstimulation. However, additional luteal phase support with hCG or gonadal steroids is required in these regimens[68] and this approach is not compatible with GnRH agonist administration in the follicular phase.

The combined use of GnRH agonists and gonadotrophins does not appear to be associated with a substantial difference in complications when compared with more traditional ovulation induction regimens. As more follicles tend to mature, at least in the long regimens, a greater risk of ovarian hyperstimulation may exist.[69]

Chromosomal anomalies are not increased[70] and cryopreserved embryos are not different[71] in GnRH agonist exposed patients. Abortion rates appear to be lower[52] or unaffected[54] by GnRH agonists. A lower rate of early miscarriage appears to be particularly evident in endometriosis patients undergoing *in vitro* fertilisation after 6 months of GnRH agonist treatment.[72] Although it was suggested that exposure to GnRH agonists in monkeys during early pregnancy may be associated with abortion and infant abnormalities,[73] accidental GnRH agonist administration in human pregnancy is usually uneventful.[74] Depot GnRH agonist use that provides measurable drug concentrations in early pregnancy is not associated with an increased abortion rate.[75]

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In conclusion, GnRH agonists improve pulsatile GnRH and gonadotrophin ovulation induction and increase the overall success of assisted reproduction procedures. Thus, use of these agents in these applications should be expected to grow.

4.3 Uterine Leiomyoma

The profound hypoestrogenism induced by GnRH agonists rapidly affects uterine and leiomyoma size.[76] Profound reductions of uterine/leiomyoma volume have been reported in almost 250 patients in several recent large studies,[77,78] some of which were conducted in a double-blind, placebo-controlled fashion.[79,80] The most profound volumetric decrement occurs within 3 to 4 months from the initiation of treatment. Additional minor (usually not statistically significant) decrements continue up to the sixth month of therapy.

Leiomyoma return to pretreatment volume within a few months after the discontinuation of the GnRH agonist.[79] In addition, myomas may recur even after surgical removal. It was suggested in one study that GnRH agonist treatment immediately before surgery may favour tumour recurrence.[81] However, the relationship between GnRH agonist administration and postsurgical recurrence of myomas was not confirmed by Friedman et al.[82]

The administration of low dose GnRH agonists appears to be as effective as more standard dosages.[78] Both the subcutaneous and intranasal routes of GnRH agonist administration are effective.[77] Efficacy of treatment is usually monitored with pelvic ultrasound. In addition, myoma shrinkage during GnRH agonist has been confirmed by nuclear magnetic resonance.[83-85]

Although GnRH binding sites were identified in myoma tissue,[86] the therapeutic effect of GnRH agonists is likely related only to hypoestrogenism. The degree of myoma shrinkage was found to be inversely related to estradiol concentrations at the twelfth week of GnRH agonist treatment.[87] Myomas obtained at surgery during GnRH agonist treatment show signs of decreased proliferative activity[88] and of ischaemic injury and cellular atrophy.[89]

Complications reported during GnRH agonist treatment in patients with leiomyoma include sepsis after expulsion of necrotic fibroids[90] and a pseudo–Meigs' syndrome with ascitis.[91] Particular care should be exercised in the differential diagnosis between leiomyoma and leiomyosarcoma since some patients with malignant uterine tumours are bound to be considered for GnRH agonist treatment.[92-94]

The addition of steroids to GnRH agonists has been tested to minimise GnRH agonist–related adverse effects such as hot flashes, lipoprotein derangements and bone loss. Concomitant supplementation with medroxyprogesterone acetate (MPA) prevented uterine volume reductions, thus abolishing clinical efficacy of GnRH agonists.[95,96] Conversely, the addition of conjugated estrogens and MPA beginning after 3 months of GnRH agonist permitted the maintenance of uterine volume reductions while controlling adverse effects.[20] Greater efficacy of combined estrogen/progestogen *vs* progestogen alone supplementation was more recently confirmed by Friedman et al.[19]

Because of its palliative nature and the rapid reversibility of the therapeutic effects of GnRH agonists, this form of treatment has recently become controversial. Several studies have suggested that GnRH agonists given in preparation for surgery may simplify the operative procedure, reduce bleeding and complications, or permit vaginal rather than abdominal hysterectomy.[97-100] However, the positive effects of GnRH agonist pretreatment on classical surgical procedures is likely to be marginal if any. Conversely, GnRH agonist pretreatment could be more relevant in endoscopic procedures, such as laparoscopic and hysteroscopic myomectomy, for which reduced myoma size and diminished blood loss are critical parameters for optimal treatment outcome.[101-103]

Despite the limitations of this form of treatment, it is likely that the use of GnRH agonists for leiomyoma management will increase. In addition

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to endoscopic surgery, GnRH agonist use prior to traditional surgery permits the correction of the anaemia which commonly presents in these patients[19,76] and, thus, reduces the chances of blood transfusion being needed. Blockage of menometrorrhagia may also allow these patients to be scheduled for elective rather than emergency surgery.

Although GnRH antagonists[104] and the antiprogestogen mifepristone (RU486)[105] have been reported to be capable of reducing leiomyoma size, at present, GnRH agonists are the only clinically available medication effective for the management of this condition.

4.4 Endometriosis

Endometriosis is a not uncommon disorder in women. It causes dysmenorrhoea, dyspareunia, infertility and other symptoms. Since 1982,[106] GnRH agonists have been used for the treatment of this condition.

GnRH agonists have also been shown to be effective in the management of less common or rare presentations of endometriosis, such as adenomyosis,[107,108] renal[109] and pulmonary endometriosis,[110] and in endometriosis-related ascitis and pleural effusion.[111] Outcomes of this drug regimen have often been compared with the more traditional use of danazol, a synthetic progestin with significant anabolic/androgenic activity. GnRH agonists result in more profound LH and estrogen suppression than danazol.[112] Conversely, GnRH agonists appear to be devoid of direct endometrial effects, while MPA and danazol reduce endometrial stromal cell proliferation.[113]

Both GnRH agonists and danazol induce noncyclicity and atrophy of the endometrium. Danazol is associated with marked progestational effects on endometrial glands and stroma, and mucosal hypotrophy. GnRH agonist administration results in a weakly proliferative or inactive mucosa.[114] Additional differences exist in lipoprotein concentration patterns. GnRH agonists increase but danazol lowers high-density lipoproteins (HDL) and particularly HDL cholesterol (HDL-C). Low density lipoproteins (LDL) are often increased during danazol administration.[115,116] Thus, danazol worsens the atherogenic index (cholesterol/HDL-C), while GnRH agonists are associated with a fairly normal lipoprotein profile.

Evaluation of treatment efficacy in endometriosis is cumbersome because second look laparoscopy should be performed. Measurements of serum CA125, a cell surface antigen often increased in endometriosis, proved to be unsatisfactory for monitoring treatment efficacy as this marker appears to be correlated mostly to ovarian activity.[117]

Several large studies reporting clinical and laboratory results of treatment in over 1000 patients have recently been published.[115,118-120] GnRH agonists and danazol appear to be equally effective in reducing the severity of endometriotic implants and of dysmenorrhoea. Hypoestrogenic adverse effects (hot flashes, bone loss, reduced libido) prevail during GnRH agonist administration, while anabolic/androgenic disturbances (bodyweight gain, hirsutism, lipoprotein derangements) are typical of danazol. In at least one large study, more patients on danazol than on GnRH agonists withdrew from treatment because of adverse effects.[119]

Progestogen supplementation was tested to limit adverse effects during GnRH agonist administration. MPA reduces hypoestrogenic adverse effects, but blocks the efficacy of GnRH agonists on endometriosis.[121] Norethisterone (norethindrone) coadministration appears to reduce adverse effects while maintaining GnRH agonists therapeutic efficacy. However, it is associated with a decrement of the HDL/LDL ratio similar to that caused by danazol.[122]

In the first year following treatment discontinuation, the symptoms of endometriosis tend to reappear in both GnRH agonist– and danazol-treated patients, although they seem to be less common and milder than before treatment.[120] Spontaneous fertility rates at the end of GnRH agonist therapy appear encouraging,[115,118] although the exact effect of treatment is not clear. Furthermore,

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GnRH agonist pretreatment significantly improved pregnancy rates in patients undergoing *in vitro* fertilisation.[72]

The beneficial effects of GnRH agonists on endometriosis are mostly limited to the period of its administration. However, reduction of symptoms that extends beyond GnRH agonist treatment, fewer adverse effects than danazol, and improved fertility when given before assisted reproduction techniques should result in expanded use of GnRH agonists in this disorder.

4.5 Prostate Cancer

Administration of GnRH agonists for the medical, nonsurgical management of stage D prostate cancer is well established. Most GnRH agonists are registered for this application. The availability of depot (intramuscular or subcutaneous) GnRH agonists has simplified this form of long term treatment. In the near future, depot GnRH agonists with slow-release characteristics allowing even more prolonged activity (2 or 3 months) will further enhance ease of use of this therapy.

Long term endocrine and clinical results of GnRH agonist administration are similar to those of surgical castration.[123] In contrast to surgical castration, GnRH agonist administration initially results in a markedly, albeit temporary (2 to 3 weeks), increment of gonadal androgens (flare phenomenon) that may cause transient worsening of symptoms and an increase of tumour volume. Antiandrogens such as flutamide have been used in the early period of GnRH agonist administration to counteract the flare phenomenon. The combined long term use of GnRH agonists and flutamide (complete androgen blockade) has also been advocated.[124] The rationale for this regimen is to complement gonadal steroid blockage with the abolishment of the possible stimulatory actions of adrenal androgens upon tumour tissue. Although this approach was found to be effective in slowing tumour progression and improving survival,[125] complete androgen blockade is still the subject of controversy.[123,126-128]

Personal, psychological, and economic considerations should be examined when choosing between medical treatment with GnRH agonists and surgical castration. In view of the cost of each of these procedures, it was suggested that GnRH agonist administration should be carried out first, while orchidectomy could be resorted to after 2 years of medical treatment in long term responders.[129]

4.6 Breast Cancer

Breast cancer is a frequent cause of disease and death in women. Breast cancer cells can retain sensitivity to estrogens. Thus, surgical oophorectomy was commonly performed for many years to slow tumour progression. When properly administered, GnRH agonists can reduce ovarian estrogens to castration concentrations and have been used in the last decade in the management of this condition.[22]

More recently, larger studies have confirmed that GnRH agonists can achieve an objective response in 35 to 40% of patients with premenopausal breast cancer.[130,131] Better response was associated with an estrogen receptor-positive status, although some patients with receptor-negative tumour cells also appeared to benefit from GnRH agonist administration. These results are similar to those obtained with surgical oophorectomy.[132]

The concept of complete endocrine blockade used in prostate cancer is also being tested in breast cancer, with the combined administration of GnRH agonists and the antiestrogen tamoxifen. Preliminary results, however, indicate that the addition of tamoxifen does not appear to enhance GnRH agonist efficacy in terms of survival rates, although the overall time to disease progression in patients treated with the combined regimen is prolonged.[133]

Finally, the identification of GnRH receptors on breast cancer cells and the *in vitro* efficacy of GnRH agonists to inhibit the growth of cancer cells suggested that these compounds may also be effective in the treatment of postmenopausal patients.[22,134] Unfortunately, other studies did not

support a clinical efficacy of GnRH agonists in patients with postmenopausal breast cancer.[135] The use of GnRH agonists should, thus, be reserved for the management of patients with premenopausal breast cancer.

4.7 Other Types of Cancer

4.7.1 Ovarian Cancer

Ovarian cancer is relatively common in women and late diagnosis accounts for the elevated mortality of this condition relative to other gynaecological cancers. It was suggested that GnRH agonists affect ovarian cancer development through a reduction of endogenous gonadotrophins that may stimulate cancer development[136] or a direct action on cancer cells.[137] Preliminary studies suggested that GnRH agonists may be effective in inducing remissions in ovarian cancer patients.[138] However, more recent trials failed to substantiate the therapeutic efficacy of GnRH agonists in ovarian cancer,[139] and determined that these agents are not better than tamoxifen in the treatment of this condition.[140]

4.7.2 Endometrial Cancer

GnRH receptors were recently identified in human endometrial cell cancer lines[141] and in biopsy specimens of human endometrial cancer.[142] A preliminary clinical study in patients previously treated with surgery, radiotherapy and progesterone demonstrated remission in 6 out of 17 patients.[143] GnRH agonist efficacy could be mediated by direct antitumour effects and/or inhibition of endogenous gonadotrophins/steroids. However, additional studies performed in a double-blind, placebo-controlled fashion will be needed to properly assess the efficacy of GnRH agonists in this condition.

4.7.3 Adenocarcinoma of the Pancreas

In 1984, it was shown that GnRH agonists decrease tumour weight and the volume of pancreas acinar and ductal carcinoma in animals.[144] In a preliminary clinical study, 5 patients with advanced adenocarcinoma of the pancreas were treated with GnRH agonists and showed some degree of improvement of their clinical condition and quality of life.[145] However, a more recent study in 7 male patients with stage III or IV pancreatic cancer failed to show any response to GnRH agonist administration.[146]

4.8 Catamenial Disorders

Catamenial disorders are defined as medical conditions that appear or are exacerbated at specific times of the spontaneous menstrual cycle (usually around menses). With their unique capacity of abolishing the hormonal fluctuations and uterine bleeding of the menstrual cycle, GnRH agonists are appealing candidates in the management of these conditions. However, advantages of this treatment and severity of each disorder should always be weighed against the adverse effects of GnRH agonists, such as vasomotor symptoms and bone loss.

4.8.1 Premenstrual Syndrome

Muse et al.[147] initially proposed the use of GnRH agonists in premenstrual syndrome and found that it was effective in reducing physical and behavioural symptoms of this condition. Later studies[148,149] confirmed the efficacy of GnRH agonists, but several patients treated for extended periods of time (>2 months) withdrew from the protocols because of the severity of GnRH agonist–induced adverse effects. Thus, GnRH agonists alone are unlikely to be useful for the long term management of premenstrual syndrome.

4.8.2 Other Medical Conditions

GnRH agonist administration was successfully tested in recurrent anaphylaxis,[150] cyclic auditory dysfunction,[151] cyclic attacks of acute intermittent porphyria,[152] and catamenial epilepsy.[153] Management of excessive menstrual bleeding following liver transplantation[154] is of particular interest because the severity of the medical problem overrides concerns regarding GnRH agonist–related adverse effects, and because this approach avoids the need for contraceptive methods that are contraindicated in this condition (oral contraceptives, intrauterine devices) since no con-

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ception can occur when ovulation is suppressed by GnRH analogues.

4.9 Hyperandrogenism

Hyperandrogenism in women can be related to excessive ovarian and/or adrenal androgens. GnRH agonists were found to be highly effective in reducing ovarian hyperandrogenism without affecting adrenal hormone secretion.[22,155] LH and androgens rapidly return to pretreatment concentrations when GnRH agonists are discontinued in PCOS[156,157] and few patients ovulate spontaneously.[157] Hirsutism[22] and acne[158,159] markedly improve during GnRH agonist treatment. Nevertheless, the availability of alternative effective medications, such as cyproterone and spironolactone, better suited for long term management of hirsutism, has markedly limited the therapeutic usefulness of GnRH agonists in this condition.

A combination of GnRH agonist and estrogen/progestogen replacement combination regimen was recently reported to be more effective and associated with fewer adverse effects than GnRH agonists alone for the treatment of hirsutism[21]. However, given the elevated cost of long term GnRH agonist administration, greater efficacy of this approach compared with the more traditional and far less expensive steroid-only therapy will have to be demonstrated. GnRH agonists were also used to characterise hyperandrogenic patients,[160] to identify the precise source (ovarian or adrenal) of excessive androgen secretion,[161] and to assess the relationship between hyperinsulinaemia and hyperandrogenism in PCOS.[162,163]

4.10 Menometrorrhagia

Severe dysfunctional uterine bleeding can, among other things, be related to uterine abnormalities such as leiomyoma or deranged ovarian hormone secretion.[164] GnRH agonists can affect these conditions and have been used for the management of menorrhagia and metrorrhagia.

In addition to the reduction of excessive menstrual bleeding in patients with blood clotting disorders and liver transplant,[154] intranasal and depot GnRH agonists were successfully given to women with menometrorrhagia.[165,166] Unfortunately, results in few patients have been reported so far.

A combination of GnRH agonist and estrogen/progestogen combination treatment to limit adverse effects was tested with good results by Thomas et al.[167] However, this approach could not distinguish the specific role of each medication in improving symptomatology. Additional more extensive studies will be required to better characterise the efficacy and exact role of GnRH agonists in this clinical application.

4.11 Other Applications

4.11.1 Prostatic Hypertrophy

Elderly men often experience prostate enlargement that may cause urine flow impairment or obstruction. Testosterone, dihydrotestosterone (androstanolone), and possibly estrogens can stimulate prostatic tissue. GnRH agonists would, thus, appear to be good candidates to improve prostatic hypertrophy. GnRH agonist administration significantly reduced prostate volume, although only by about 25 to 35%, and discontinuation of treatment resulted in the return of the organs to their initial size by 3 to 6 months.[168,169] Furthermore, only about one-third of patients experienced objective improvement of their condition.[168,169]

The availability of surgical management of prostatic hypertrophy and alternative effective medical approaches associated with fewer adverse effects (finasteride) limit the usefulness of GnRH agonists in this application.

4.11.2 Fibrocystic Breast Disease

The marked hypoestrogenism induced by GnRH agonists has been exploited for the management of fibrocystic mastopathy.[170] A complete or partial response was obtained in virtually every patient. However, the typical adverse effects related to GnRH agonist administration prevent a long term applicability of this treatment in this benign disorder.

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4.1.1.3 Gonadal Protection from Chemotherapy and Irradiation

The possibility that inhibition of gonadal function before and during chemotherapy treatment may preserve reproductive potential was initially suggested by Glode et al.[171] Unfortunately, clinical trials performed in male and female patients with non-Hodgkin lymphoma,[172] Hodgkin's disease,[173] teratoma,[174] and seminoma[175] treated with chemotherapy or irradiation failed to demonstrate any protective action of GnRH agonist cotreatment.

5. Selecting Agents for Use

In principle, all GnRH agonists presently registered can be used for any of the clinical applications listed in this article. Nevertheless, absorption and pharmacodynamic characteristics of these agents may differ in relation to the route of administration and drug formulation.

As a general concept, the intranasal route of administration provides lower systemic GnRH agonist absorption than the subcutaneous route. A higher dose and more frequent administration must, thus, be used when GnRH agonists are given intranasally. Nevertheless, inadequate pituitary-gonadal suppression may still result with intranasal delivery and, for this reason, intranasal GnRH agonist formulations tend to be less frequently prescribed now than in the past.

Depot (intramuscular or subcutaneous) GnRH agonists are effective, more practical and provide better patient compliance because of their infrequent mode of administration (once every 1 or 2 months). These GnRH agonist formulations are now electively chosen for long term treatments lasting several months or years. However, not all depot GnRH agonists may be equally effective.[16] Furthermore, GnRH agonists tend to be released from depots in relevant, albeit reduced, amounts for several days following the end of theoretical efficacy of these formulations. Thus, depot GnRH agonists should not be used in applications such as ovulation induction with pulsatile GnRH and with short gonadotrophin regimens where interruption of pituitary suppression or short term efficacy are required.

6. Conclusions

The great efficacy and safety of GnRH agonists are the key factors in the success of these compounds in virtually every country in which they are commercially available. However, a sound understanding of the pathophysiology of the condition to be treated is essential to achieving optimal results.

Prompt reversibility of pituitary-gonadal suppression at the time of drug discontinuation is a fundamental and advantageous characteristic of GnRH agonists. Nevertheless, this feature also underlies the essentially palliative effects of GnRH agonists in both benign and malignant reproductive disorders (table II). Conversely, in ovulation induction GnRH agonists may have a more fundamental effect on the physiological regulation of folliculogenesis and ovulation, but the exact role of these compounds is still controversial.

Finally, all the therapeutic actions of GnRH agonists appear to be mediated through pituitary-related hypogonadism. Thus, clinical applications that depend on a postulated action of GnRH agonists on non-pituitary tissues are unlikely to gain future relevance.

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Endocrine-Related Cancer (2000) **7** 73–83

Hormonal contraception and chemoprevention of female cancers

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Abstract

Oral contraceptive (OC) use significantly reduces the risk of endometrial and ovarian cancer, has only a minimal effect on breast cancer, but may increase the risk of cervical cancer. These effects can be readily explained in terms of the effects of OCs on cell proliferation in these tissues. This analysis suggests how a hormonal contraceptive based on a GnRH agonist plus low-dose add-back sex steroids could be made that would greatly reduce lifetime risk of breast and ovarian cancer. Such a hormonal contraceptive is also likely to significantly reduce the lifetime risk of cervical cancer. It is also likely to reduce the risk of endometrial cancer, although not to the same extent as OCs.

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Introduction

Hormonal chemoprevention of ovarian cancer was first demonstrated in the late 1970s. The chemopreventive agent was oral contraceptives (OCs; the 'Pill'). The protection achieved was clinically highly significant and dependent on the duration of use: 5 years of OC use provides a long-term reduction in risk of some 32%, and 10 years of use a reduction of some 54% (Pike & Spicer 1993). Hormonal chemoprevention of endometrial cancer was first demonstrated in the early 1980s. The chemopreventive agent was again OCs. The protection was again clinically highly significant and dependent on the duration of use: 5 years of OC use provides a long-term reduction in risk of some 46%, and 10 years of use a reduction of some 71% (Pike & Spicer 1993). These chemopreventive effects of OCs do not, however, extend to breast cancer, and a significantly increased risk of cervical cancer has been noted in OC users.

The critical direct actions of the natural ovarian hormones, estradiol and progesterone, and their synthetic analogs in OCs, on endometrial, breast and cervical cells are to affect cell division rates. OCs respectively decrease, leave effectively unchanged and appear to increase mitotic activity of endometrial, breast and cervical cells. OCs indirectly reduce the mitotic activity of ovarian epithelial cells. The effects of OCs on cancer rates in these tissues can be largely explained on the basis of these differing effects on cell proliferation.

An obvious way to attempt to extend the protective effects of OCs from endometrial and ovarian cancer to include breast and cervical cancer in addition is to look for ways of simultaneously reducing cell division in all four tissues. The design requirements of a hormonal contraceptive to achieve this are described in Table 1 and are discussed below.

Table 1 Design requirements of a hormonal contraceptive to reduce cancer risk

Organ	Design aim
Endometrium	Reduce exposure of the endometrium to 'unopposed' estrogen
Ovary	Reduce ovulation frequency and follicle stimulation
Breast	Reduce exposure of the breast to 'estrogen plus progestin'
Cervix	Reduce exposure of the cervix to 'estrogen plus progestin'

The contraceptive approach we have proposed is to block ovarian function with a gonadotropin-releasing hormone agonist (GnRHA) and to counteract the induced hypo-estrogenism with a low dose of estrogen and intermittent (12–14 days every 3–4 months) progestin (Pike *et al.* 1989, Spicer *et al.* 1993). This approach avoids having to use the high dose of estrogen–progestin of OCs to block ovulation. The main aim of this approach is chemoprevention of breast and ovarian cancer while providing a hormonal contraceptive that will be highly acceptable to most women. This approach has been demonstrated to be highly acceptable to women at high risk of breast cancer (Spicer *et al.* 1993). There is substantial evidence that this approach will reduce ovarian cancer and almost as much evidence that it will

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reduce breast cancer. Endometrial cancer protection is not achieved to the extent achieved by OCs, as OCs deliver a high daily dose of progestin with each daily dose of synthetic estrogen. The results of a recent epidemiological study, however, suggest that, as long as the duration of the intermittent progestin is at least 13 days and the estrogen dose is low, hyperplasia will not be induced and sloughing of the endometrium will be sufficient to provide significant protection against endometrial cancer (Pike *et al.* 1997).

A recent epidemiological study found that oophorectomy, even if combined with some add-back hormone replacement therapy, was associated with a sharp reduction in risk of breast cancer in women at high genetic risk of the disease (Rebbeck *et al.* 1999). These results provide strong support for this GnRHA approach to chemoprevention of breast cancer, and that the approach is equally applicable to high-risk women. These epidemiological observations are also strongly supported by the results of a recently published randomized clinical trial of using a GnRHA for treating premenopausal breast cancer patients; this trial showed a marked reduction in the occurrence of contralateral primaries in the GnRHA-treated patients (Baum 1999).

Endometrial cancer

The incidence of most non-hormone-dependent cancers rises continuously and increasingly rapidly with age; and a plot of the logarithm of incidence against the logarithm of age produces a straight line as predicted by the multi-stage theory of carcinogenesis and confirmed by modern molecular biology studies (Fig. 1). In contrast, the age-incidence curve of cancer of the endometrium shows a distinct slowing of the rate of rise at the age of menopause (top curve in Fig. 2). The key etiologic elements are therefore present in premenopausal women, but are reduced following menopause. Increasing parity also decreases endometrial cancer risk. Obesity in both pre- and postmenopausal women, and estrogen replacement therapy in postmenopausal women, significantly increases endometrial cancer risk. OC use significantly decreases endometrial cancer risk (Centers for Disease Control 1983*b*, 1987*b*, Henderson *et al.* 1983, Key & Pike 1988*a*).

These risk factors are all explained by the mitogenic action of estrogen in the absence of progestin (so called 'unopposed estrogen') in stimulating endometrial-cell division. During a normal menstrual cycle, endometrial-cell mitotic activity peaks during the early follicular phase, when serum estradiol levels are approximately 50 pg/ml; further increases in unopposed estradiol concentration do not appear to increase the mitotic rate (Fig. 3*a* and *b*). Following ovulation, serum progesterone rises and endometrial-cell proliferation ceases despite continued estradiol levels in excess of 50 pg/ml. At low serum estradiol concentrations (5 pg/ml), as occur in slender postmenopausal women, endometrial-cell mitotic activity is very low. There is thus a dose–response relationship between unopposed estradiol concentration and endometrial mitotic rate in the range from around 5 to around 50 pg/ml.

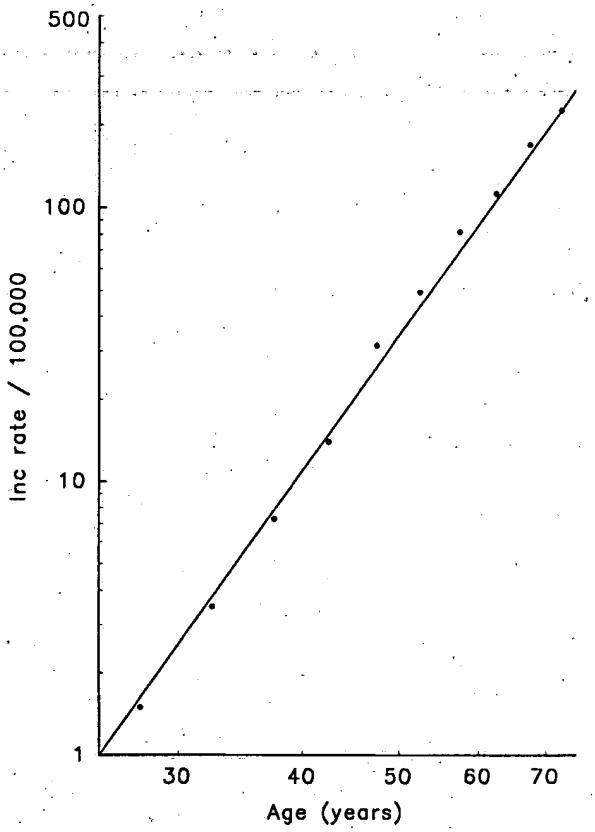

Figure 1 Age-specific incidence (Inc) rates for colorectal cancer in US white females 1969–1971 (Cutler & Young 1975).

Early menopause reduces endometrial cancer risk by reducing the serum unopposed-estrogen concentration to a very low level. Progesterone effectively opposes estrogen throughout pregnancy, so increasing parity is associated with decreasing risk. In premenopausal women, obesity increases risk through the increased anovulation of such women, and their serum estradiol level is sufficiently high to cause maximal endometrial-cell proliferation. In obese postmenopausal women, unopposed-serum estrogen is increased and sex-hormone-binding globulin is decreased, so that there is an increase in bioavailable estrogen. Estrogen replacement therapy increases serum unopposed estrogen.

OCs contain an estrogen and a high-dose progestin; endometrial cells are, thus, exposed to unopposed estrogen only during the 7 days in 28 during which the OC is not taken, and the endogenous estrogen level during these 7 days remains quite low. Meta-analysis of the population-based epidemiological studies shows a reduction in endometrial cancer risk of around 11.7% per year of OC use (Pike &

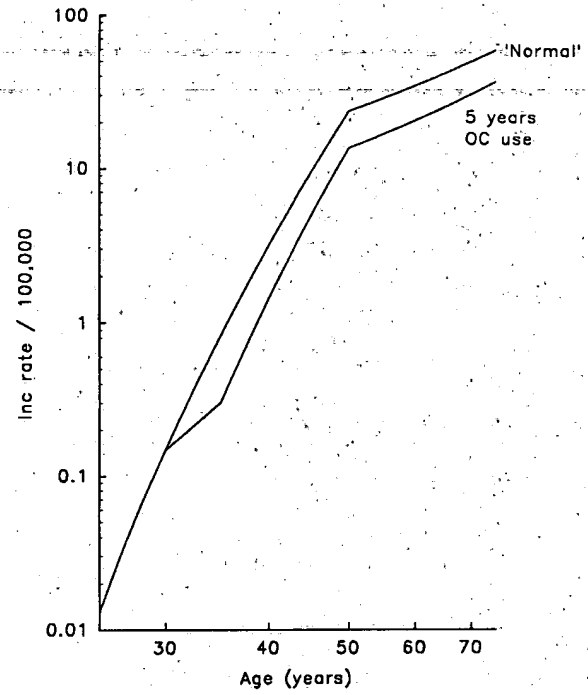

Figure 2 Age-specific incidence (Inc) rates for endometrial cancer in women in the Birmingham region of the UK 1968–1972 (Waterhouse *et al.* 1976). Note: this curve is derived from UK data because US data are severely biased owing to the high oophorectomy rate in the USA. The lower curve is for such women using OCs for 5 years from age 30 to age 35.

Spicer 1993), i.e. a 46% reduction per 5 years of OC use. The lower curve in Fig. 2 translates these results to the age-incidence curve of endometrial cancer; the curve shows the effect of 5 years of OC use from age 30 to age 35. The slope of the curve is much reduced during the time OCs are used and then increases again once OC use is stopped, but the protection gained continues after stopping.

Ovarian cancer

The age-incidence curve of cancer of the ovary, like the age-incidence curve for cancer of the endometrium, shows a distinct slowing of the rate of rise at the age of menopause (top curve in Fig. 4). Again the key etiologic elements are present in premenopausal women, but are reduced following menopause. The other major factor determining ovarian cancer risk is parity; increasing parity decreases risk, and increasing duration of breast feeding also decreases risk. OC use significantly decreases ovarian cancer risk (Casagrande *et al.* 1979, Willett *et al.* 1981, Centers for Disease Control 1983*a*, 1987*a*, Risch *et al.* 1983). These protective factors interrupt ovulation and reduce intra-ovarian hormone levels, in particular, intra-ovarian estrogen levels.

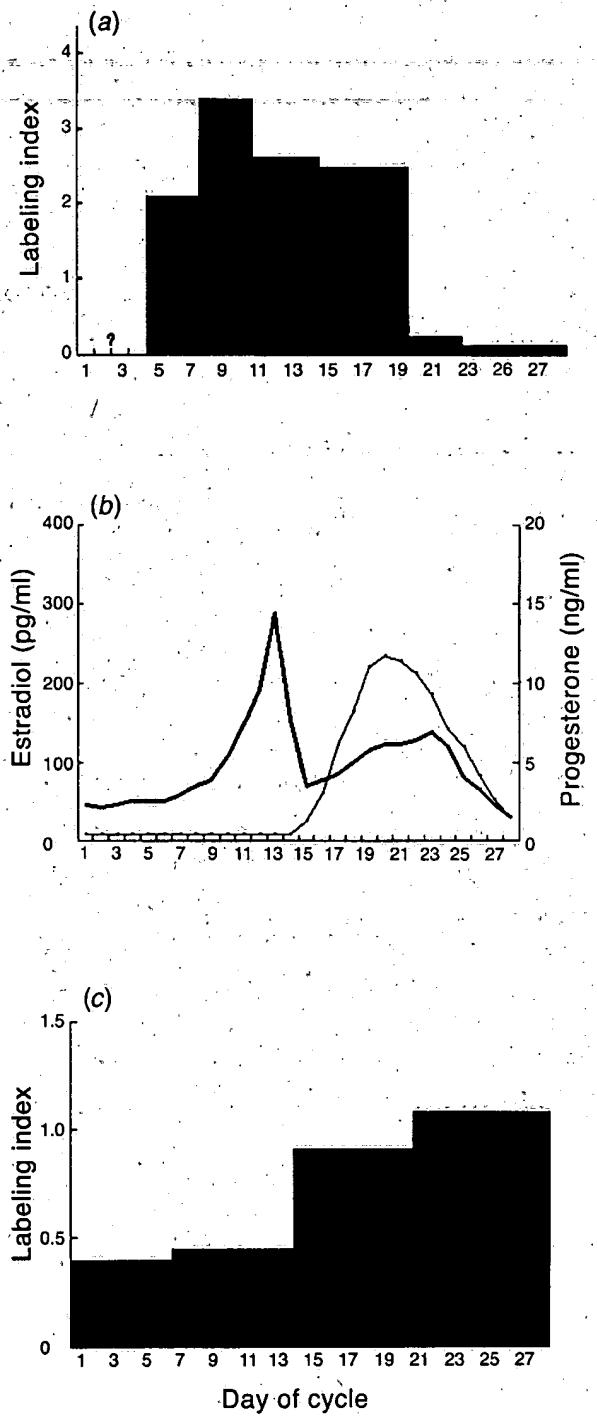

Figure 3 (*a*) Endometrial-cell labeling index (mitotic rate) by day of cycle (day 1 is the first day of menses and a 28-day cycle is assumed with ovulation on day 14) (Ferenczy *et al.* 1979). (*b*) Serum concentrations of estradiol (heavier line) and progesterone (lighter line) by day of cycle (Goebelsmann & Mishell 1979). (*c*) Breast-cell labeling index (mitotic rate) by day of cycle in parous women (Anderson *et al.* 1989).

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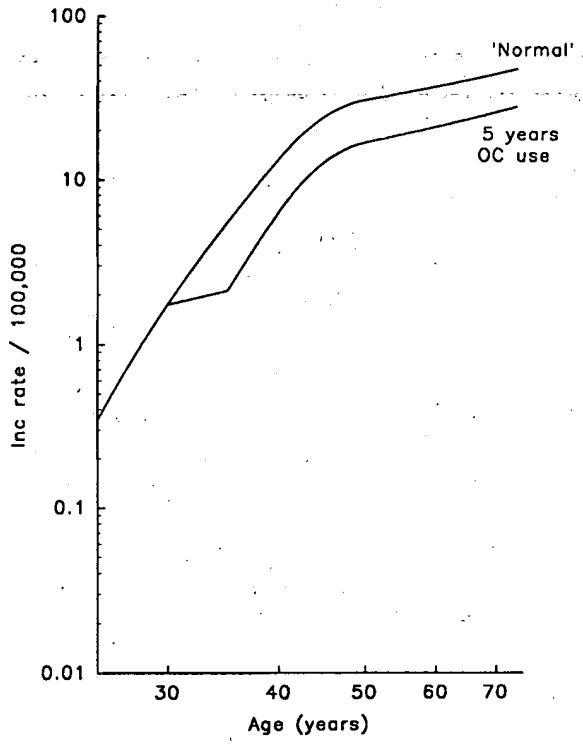

Figure 4 Age-specific incidence (Inc) rates for ovarian cancer in women in the Birmingham region of the UK 1968–1972 (Waterhouse *et al.* 1976). Note: this curve is derived from UK data because US data are severely biased owing to the high oophorectomy rate in the USA. The lower curve is for such women using OCs for 5 years from age 30 to age 35.

The incessant ovulation hypothesis for ovarian cancer proposes that ovarian cancer risk is essentially determined by the increased proliferative activity of the ovarian surface epithelium required to accomplish repair of the surface after each ovulation (Fathalla 1971, 1972). The incessant ovulation hypothesis provides a quantitative explanation of the decreasing risk found with pregnancies, breast feeding and OC use (Pike 1987), but cannot explain the much reduced incidence rates of ovarian cancer found until quite recently in low-risk Asian countries. Until quite recently the age-specific incidence rates of ovarian cancer were very much lower in China and Japan than in the USA. For example, in 1970, before the widespread use of oral contraceptives began to cause a significant fall in the incidence of ovarian cancer in young women in the USA, the ovarian cancer incidence rate (per 100 000 females) at ages 45–54 was 30.8 in the USA, compared with 6.4 in Japan. This 4.8-fold difference cannot be explained on a simple incessant ovulation hypothesis. Japanese women born around 1920 had menarche some 1.7 years later than US white women born around that time, they had 0.5 more live births, and their average menstrual cycle may have been as much as 2 days longer (Grove *et al.* 1968, Hoel *et al.* 1983, Japan Statistical Yearbook 1998). These differences can only account for a 1.7-fold lower rate in the Japanese on the incessant ovulation hypothesis (Pike 1987). The observed difference in rates can, however, be readily accounted for on a hypothesis that includes intra-ovarian estradiol levels as a risk factor. Estradiol at an intra-ovarian concentration is a potent mitogen to ovarian cystadenoma cells (MP Luo, MC Pike, W Zheng, MR Stallcup & L Dubeau, unpublished observations) and women in low-risk Asian countries have serum estradiol levels some 25% lower than those of US women (Goldin *et al.* 1986, Bernstein *et al.* 1990, Key *et al.* 1990). Such a reduction is quite sufficient to account for the remaining difference in ovarian cancer rates between Japan and the USA (Pike 1987).

OC use blocks ovulation and markedly reduces intra-ovarian estrogen levels. Meta-analysis of population-based epidemiological studies shows a reduction in ovarian cancer risk of approximately 7.5% per year of OC use (Pike & Spicer 1993), i.e. a 32% reduction per 5 years of OC use. The lower curve in Fig. 4 translates these results to the age-incidence curve of ovarian cancer; the curve shows the effect of 5 years of OC use from age 30 to age 35. The slope of the curve is much reduced during the time OCs are used and then increases again once OC use is stopped, but the protection gained is only slightly reduced after stopping and again the protective effect should be lifelong (as it is for parity).

OC use has been found to be equally protective against hereditary (mutBRCA1/2) ovarian cancer (Narod *et al.* 1998).

Breast cancer

The age-incidence curve of cancer of the breast, like cancer of the endometrium and ovary, shows a distinct slowing of the rate of rise at the age of menopause (Fig. 5). Again the key etiologic elements are present in premenopausal women, but are reduced following menopause. Breast cancer risk decreases with increasing age at menarche, and obesity during the postmenopausal years increases breast cancer risk, but obesity during the premenopausal years actually reduces risk (Key & Pike 1988*b*). Use of menopausal estrogen replacement therapy only marginally increases breast cancer risk, but use of estrogen plus progestin therapy in the postmenopausal period increases breast cancer risk substantially (Magnusson *et al.* 1999, Ross *et al.* 2000, Schairer *et al.* 2000).

During the menstrual cycle, breast-cell proliferation is lowest during the follicular phase and then increases some twofold in the luteal phase (Fig. 3*c*; Pike *et al.* 1993). This suggests that estradiol induces cell division and that the combined effect of estradiol and progesterone together is considerably greater – the estrogen plus progestin hypothesis.

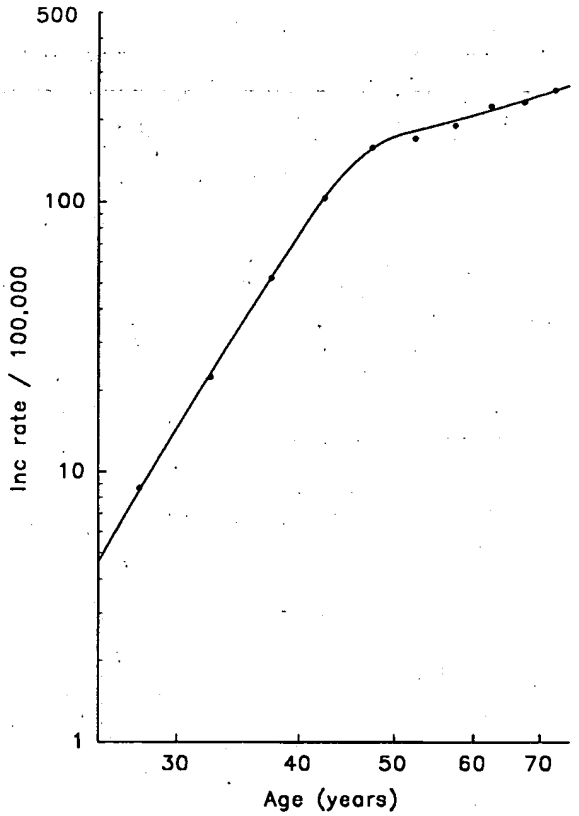

Figure 5 Age-specific incidence (Inc) rates for breast cancer in US white females 1969–1971 (Cutler & Young 1975).

In the postmenopausal period, when estradiol levels are low, and progesterone is absent, breast-cell proliferation is very low. The protective effects of late menarche and early menopause are thus readily explained by this hypothesis. The contradictory effects of obesity are also predicted by this hypothesis. The increased anovulation associated with premenopausal obesity will decrease breast exposure to both estradiol and progesterone; after menopause, the decreased risk associated with premenopausal obesity is gradually eliminated and an increased risk finally achieved by the increased bioavailable estrogen levels associated with postmenopausal obesity. The reason that risk is only slightly increased by estrogen replacement therapy is the relative low dose of estrogen used in estrogen replacement therapy (Key & Pike 1988*b*) and the absence of a progestin. The addition of a relatively high-dose progestin to estrogen replacement therapy is predicted to increase the risk substantially. This is precisely what is observed; the three recent large-scale population-based epidemiological studies (Magnusson *et al.* 1999, Ross *et al.* 2000, Schairer *et al.* 2000) suggest that the added risk is increased from about 10% per 5 years of use with estrogen replacement therapy to approximately 30% per 5 years of estrogen plus progestin replacement therapy. These results are strongly supported by the findings of greatly increased breast-cell proliferation in postmenopausal women on estrogen plus progestin replacement therapy (Hofseth *et al.* 1999), and the findings in a randomized trial of greatly increased mammographic densities with such use of estrogen plus progestin (Greendale *et al.* 1999).

Studies of OC use and breast cancer have found either no effect or a slight increase in risk. This is entirely consistent with the estrogen plus progestin hypothesis. OCs contain an estrogen and a progestin. OCs inhibit gonadotropin secretion, thus reducing ovarian steroidogenesis to very low levels, but the ovarian steroid loss is compensated for by the synthetic estrogen and progestin of the OC. One would predict that breast-cell proliferation in women taking OCs would be less than, equal to, or greater than that observed during a normal menstrual cycle, depending on the dose of estrogen and progestin in the particular OC. Direct observational studies of breast-cell proliferation in women taking OCs suggest that the total breast cell proliferation is very similar over an OC cycle and a normal menstrual cycle (Anderson *et al.* 1989, Williams *et al.* 1991). These results predict that breast cancer risk should not be substantially affected by OC use, as is observed.

Cervical cancer

The incidence of invasive cervical cancer fluctuates widely in different (birth) cohorts of women. It is also markedly reduced by cervical cancer screening. To note the true age-incidence distribution of the disease it is necessary to use cohort-specific data for time-periods before cervical cancer screening (Pap screening) was common. Such an analysis was conducted by Cook & Draper (1984) for cervical cancer mortality. Figure 6 shows the age-specific mortality curve that can be derived from their results. Inspection of some age-incidence data from the same time-periods as studied by these authors show that this curve reasonably accurately reflects the related age–incidence curve of invasive cancer with a lag period of a few years. Figure 6 strongly suggests that hormones may also play an important role in the development of cervical cancer.

Konishi *et al.* (1991) found that the mitotic rate of cervical cells fluctuated during the menstrual cycle in a manner similar to that of breast cells; the cervical mitotic rate was 1.8-fold greater in the luteal phase that in the follicular phase and was much reduced in postmenopausal women. The mitotic rate during pregnancy was as high as in the luteal phase of the menstrual cycle. One would therefore predict that menopause would protect against cervical cancer (as is suggested by Fig. 6), that pregnancies would increase risk (there is considerable evidence of this), and that, since OCs contain both an estrogen and a progestin, the effect of

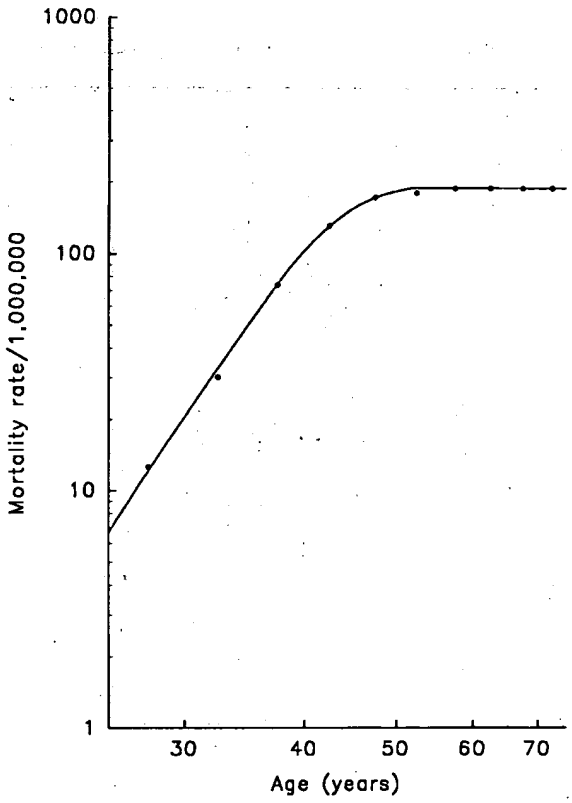

Figure 6 Age-specific cohort mortality rates for cervical cancer in UK females (Cook & Draper 1984).

the OCs would depend on the estrogen–progestin dose, much as it does for breast cancer.

OC use and cervical cancer is unusually difficult to study epidemiologically because there is a positive association of OC use with the frequency of Pap screening, and in any particular community there may be a positive association between OC use and the very significant sexual risk factors for cervical cancer.

The positive association of OC use with the frequency of Pap screening inevitably produces a positive association of carcinoma *in situ* (CIS) of the cervix with OC use whether or not there is a true association, i.e. an association independent of Pap screening history. Studies of OC use and cervical CIS must therefore adjust for Pap screening history; and, in particular, positive studies which do not do this are not interpretable. In contrast, the positive association of OC use with the frequency of Pap screening will downwardly bias any association of OC use with invasive cervical cancer, since the natural history of such cancers will be affected by Pap screening, i.e. they will tend to be picked up by screening at a premalignant stage and so never reach an invasive stage. Studies of OC use and invasive cervical cancer must therefore also adjust for Pap screening history; and, in particular, negative studies which do not do this are not interpretable.

A positive association of OC use with sexual history risk factors for cervical cancer will inevitably produce a positive bias in the observed associations of OC use with both cervical CIS and invasive cervical cancer. Studies of OC use and cervical CIS and invasive cervical cancer must therefore adjust for sexual history; and, in particular, positive studies which do not do this are not interpretable.

There are three population-based studies of OC use and invasive cervical cancer in which adjustment was made for history of Pap screening and sexual history (Brinton *et al.* 1986, Peters *et al.* 1986, Irwin *et al.* 1988). All three studies found evidence of an increased cervical cancer risk in OC users, although only the study of Brinton *et al.* (1986) had a substantial amount of data on OC use, and only the results of this study were statistically significant. A meta-analysis of the three studies showed an increase in invasive cervical cancer risk of 3.6% per year of OC use (Pike & Spicer 1993).

There is a clear need for further studies of the relationship of hormones to cervical cancer risk.

Cancer prevention by design

Table 1 summarized the design requirements of a hormonal contraceptive to reduce the risks of endometrial, ovarian, breast and cervical cancer based on the above analysis of the hormonal etiology of these cancers. OCs fulfill the design requirements for the prevention of endometrial and ovarian cancer. OCs do not provide protection against breast or cervical cancer, because OCs deliver estrogen plus progestin to the breast and cervix in quantities sufficient to replace the action of the natural estrogen plus progesterone of the normal menstrual cycle. The dose of sex steroids in present-day OCs is close to the lowest dose possible while still maintaining their contraceptive effect of preventing ovulation.

OCs are designed to achieve two separate goals. The first is to prevent ovulation, and the second is to counteract the effects of the hypo-estrogenemia caused by the blocking of ovarian function associated with the first goal. The progestin component of OCs has a vital role in suppressing ovulation, but it only has a minor role (this with respect to bone metabolism) in dealing with the associated hypo-estrogenemia. The lowest estrogen dose in conventional OCs is 30 μg ethinyl-estradiol. If the first goal of OCs, i.e. preventing ovulation, could be achieved by some other means, could the hypo-estrogenemia be countered with a lower dose of estrogen? This issue can be addressed by considering the dose of estrogen required to control menopausal hypo-estrogenemia, in particular hot flushes and adverse changes in serum cholesterol and calcium balance. The dose of ethinyl-estradiol required as estrogen replacement therapy has not been studied intensively, but the studies that are available suggest that the required dose is in

the 5–15 μg range (Spicer *et al.* 1991), i.e. at most half the dose used in current low-dose OCs.

GnRHAs, when given chronically, inhibit pituitary release of follicle-stimulating hormone and luteinizing hormone, reversibly inhibit ovulation and reduce ovarian sex-steroid production to postmenopausal levels. Thus the reversible ovulation-inhibiting function of OCs can be achieved by using a GnRHA. This enables concentration to be addressed solely on finding the combination of add-back sex steroids of greatest benefit to the user's health. As noted above, a daily dose of approximately 10 μg ethinyl-estradiol appears likely to be all the estrogen needed. Such a GnRHA plus low-dose estrogen contraceptive will achieve the design requirements for hormonal chemoprevention of ovarian, breast and cervical cancer. However, such a regimen will not prevent endometrial cancer, and some progestin is needed to control any endometrial hyperplasia which may be caused by the unopposed estrogen. Some recent studies of the effects of estrogen plus progestin replacement therapy on endometrial cancer risk suggest that prevention of endometrial cancer can be achieved by adding progestins for 13 days every third or fourth 28-day cycle of the GnRHA plus low-dose add-back estrogen regimen (see below). This amount of progestin should not affect the protective effect of the regimen against ovarian cancer as there is no evidence that progestins increase epithelial-cell division in the ovary. The predicted protection against ovarian cancer using the prototype contraceptive should be at least as great as is observed with OCs; i.e. a 32% lifelong reduction if used for 5 years, and a 69% reduction if used for 15 years. Greater reductions (41% and 84%) are likely (Pike *et al.* 1989). Any progestin is predicted to somewhat reduce the protective effects against breast and cervical cancer (but see below). We estimated that such an intermittent progestin regimen will reduce lifelong breast cancer risk by 31% if used for 5 years, 53% if used for 10 years, and 70% if used for 15 years (Spicer *et al.* 1991). The effect on cervical cancer risk is difficult to predict, but the reduced steroid dose, in particular the reduced progestin dose, is likely to produce less proliferative stimulation of the cervix than normal ovulatory cycles and hence a reduction of cervical cancer risk.

Required progestin

Unopposed estrogen replacement therapy (at the doses usually prescribed in the USA) produces significant endometrial-cell proliferation (King & Whitehead 1984). The progestin medroxyprogesterone acetate (MPA) at 5 mg/day reduces such cell proliferation to effectively zero within 6 days despite continued estrogen (Lane *et al.* 1986). However, such short-duration progestin use does not completely remove the risk of hyperplasia. Paterson *et al.* (1980) found with conjugated estrogen at 1.25 mg/day, given for 21 days per 28-day cycle, that the incidence of hyperplasia was 21.0 (per 1000 woman-months) when no progestin was used. The incidence declined to 4.0 when a progestin was used for the last 5–7 days and to 1.3 when used for the last 10 days. The incidence of hyperplasia was zero when the progestin was used for 13 days. The latter is also the number of days recommended by King & Whitehead (1984).

If endometrial cell proliferation in the basalis (stem-cell) layer was the key to increased endometrial cancer risk from estrogen replacement therapy, then there would still be a substantial increased risk even with 13 days of progestin, since there would still be unopposed estrogen for 12-15 days per treatment cycle. However, in our large epidemiological case–control study of endometrial cancer, we found only a small reduction of the estrogen replacement therapy-induced risk from 7 days of progestin, but a complete abolition of the increased risk with 10 or more days of progestin (Pike *et al.* 1997). A simple cell-proliferation model for endometrial cancer is clearly untenable.

Flowers *et al.* (1983) found 7 days of progestin did 'not cause all the endometrium to desquamate to the basalis layer . . . (only) 40 to 50% of the functional layer . . . was lost'. If these functionalis cells are susceptible to cancer, and a greater proportion of such cells are lost with longer progestin therapy, this would provide an explanation for the sharp distinction between 7 and 10 days of progestin. This would also be completely in line with the virtual complete abolition of hyperplasia with a 10-day progestin regimen (Paterson *et al.* 1980). It would also be consistent with the observation of pathologists that early stage tumors often appear to have arisen in the functionalis. If the extent of endometrial shedding in a normal menstrual cycle was no more than after a 7-day course of progestin, this would explain the sharp rise with age of endometrial cancer rates in premenopausal women even in countries where obesity is uncommon, so that obesity-related anovulation is not an explanation. There do not appear to be any data addressing this possibility.

Progestins need to be delivered to the endometrium in a manner that will be associated with low serum progestin levels so as to have a minimal effect on the breast. A vaginal (Miles *et al.* 1994, Fanchin *et al.* 1997) or direct endometrial (Shoupe *et al.* 1991) route of administration is optimal in this sense. The vaginal route provides a high endometrial progestin level with very low serum levels. The direct endometrial route of administration with an intra-uterine device has even lower serum progestin levels. If these routes of administration are unacceptable to a woman, then giving progestins for 13 days every 3–4 months may provide satisfactory protection of the endometrium with proportionally less effect on the breast than monthly administration. Two clinical trials of administering 10 mg MPA/day for 14 days every 3 months have been published in which the dose of conjugated estrogen was 0.625 mg/day (Ettinger *et al.* 1994, Williams *et al.* 1994). Both studies suggest that this approach may be satisfactory with such

low-dose estrogen. In a small 2-year study we had good results with administration of MPA every fourth month. The above discussion on the shedding of the functionalis may be the explanation for these good results which could also be predicted from the earlier work of Schiff *et al.* (1982). More studies of these approaches are urgently needed.

The above discussion is based on the use of a low-dose estrogen regimen (0.625 mg/day conjugated estrogen as is most commonly prescribed in the USA; Pike *et al.* 1997). When a 2 mg/day estradiol valerate dose of estrogen was used, every 3-month administration of progestin did not control hyperplasia (Cerin *et al.* 1996) nor was monthly cyclical progestin sufficient to completely obviate any increased endometrial cancer risk (Weiderpass *et al.* 1999). We would hypothesize that this was a higher endometrial estrogen-dose effect but have seen no data on this. Studies to elucidate these contradictory findings are urgently needed.

Some results suggesting validity of predictions

Rebbeck *et al.* (1999) studied 'a cohort of women with ... BRCA1 mutations, ... who underwent bilateral prophylactic oophorectomy but had no history of breast or ovarian cancer and had not had a prophylactic mastectomy'. Control subjects were as above but had not undergone oophorectomy. The oophorectomized women had a 47% overall reduction in breast cancer risk and a 67% reduction in incidence 5 or more years after surgery. This reduction was restricted to women whose surgery was before age 50, suggesting that the reduced breast cancer risk was a direct result of the reduced ovarian steroid levels. Hormone replacement therapy 'did not negate (this) finding ...'. This result lends strong support to our predictions that blocking ovarian function with a GnRHA, even if low-dose add-back sex steroids are used, will significantly reduce breast cancer risk. The reductions in risk observed in this study are even greater than predicted above and very close to that seen with tamoxifen.

Our presumption that GnRHA use is equivalent to oophorectomy is strongly supported by the results of the ZIPP randomized trial (Baum 1999). In this trial the GnRHA, depot Zoladex, was given to premenopausal breast cancer patients, and a 40% reduction in contralateral disease was observed (Baum 1999).

We carried out a small randomized trial of such a GnRHA, depot Lupron, plus add-back estrogen plus progestin (GEP) regimen in women at high risk of breast cancer (Spicer *et al.* 1994). The regimen we used is shown in Table 2.

Mammographic densities of women on the contraceptive regimen were quite dramatically decreased after 1 year on the regimen (Fig. 7) (Spicer *et al.* 1994). This is precisely what happens at menopause and, as we have noted, early menopause is associated with a much reduced risk of breast cancer. The statistically highly significant reductions in mammographic densities at 1 year suggest that the aim of the regimen to reduce breast cancer risk has been accomplished. In an editorial accompanying the report, Feig (1994) suggested this regimen could also lead to a greatly improved efficacy of screening mammography in young women.

Table 2 Pilot GEP contraceptive regimen

Agent and administration	Rationale
GnRHA	
Leuprolide acetate depot (Lupron Depot)	Prevent ovulation, and ovarian sex-steriod production
Estrogen	
Conjugated estrogens (0.626 mg/day p.o.; Premarin) 6 days out of 7	Prevent bone mineral loss Prevent possible rise in cardiovascular disease risk Prevent menopausal symptoms Prevent urogenital atrophy
Progestogen	
MPA (10 mg/day p.o.; Provera) 13 days every fourth 28-day cycle	Prevent endometrial hyperplasia

Menopause is associated with reduced breast-cell mitotic activity, and we believe that the associated decreased mammographic densities reflect this. The relative amounts of fibrous and adipose tissue are what determine the appearance of the mammographic image. Increased fibrous tissue equates to increased mammographic densities. Since estrogen and progesterone receptors in the breast appear to exist only in epithelial cells, the reduced sex-steroid levels of postmenopausal women are likely to affect fibrous tissue secondarily to their effect on epithelial cells.

Women on the regimen had significantly fewer 'symptoms'. This was mainly due to the sharp reduction of the symptoms associated with premenstrual syndrome (Spicer *et al.* 1993). Unscheduled bleeding or spotting was infrequent and decreased with time on the regimen. However, despite the use of an estrogen dose which is known to prevent loss of bone mineral density (BMD) in normally postmenopausal women, a small (2–3%) loss of spinal and femoral BMD was seen in the women on the GEP regimen at 1 year. The reason for this loss of BMD appears to be inhibition of ovarian androgen production. Women on treatment had a 62% drop in non-sex-hormone-binding globulin-bound testosterone. In contrast, during the early natural postmenopausal period testosterone levels are stable. This provides an explanation of why the estrogen dose we used has been found adequate for preventing bone loss in naturally menopausal women, but not in our volunteers. The addition of non-oral testosterone to the regimen simply to replace that lost by the action of the GnRHA should eliminate this problem, and is currently undergoing clinical trial.

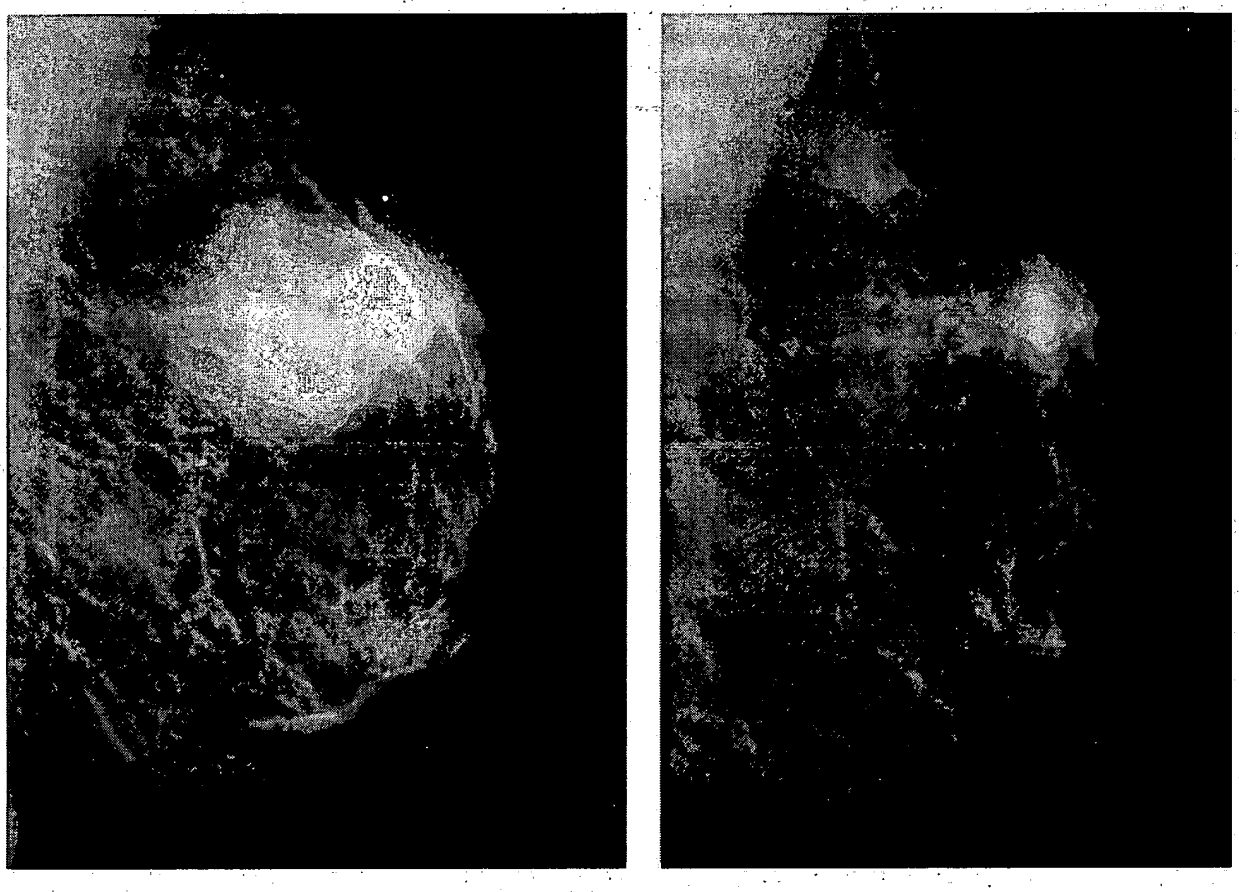

Figure 7 Mammogram at baseline and of the same breast after 1 year of use of the GEP regimen (Spicer *et al.* 1994).

Declaration of interest

The authors are associated with Balance Pharmaceuticals, Inc.; a company set up to develop the GnRHA plus the low-dose add-back regimen discussed here.

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ARTICLES

Luteinizing hormone releasing hormone agonist for contraception in breast feeding women

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During the period of lactation there is a need for a reliable method of contraception since the suppressive effects of lactation on ovulation decline as the duration of breastfeeding is decreased. The aim of this study was to establish that chronic treatment with a LHRH agonist would prevent ovulation throughout the period of lactation and to evaluate the effects of the treatment on estrogen production, bleeding patterns, and nursing practice. Starting 6 weeks postpartum, nine mothers took 300 micrograms LHRH agonist (buserelin), intranasally once daily for the remainder of the duration of breastfeeding [216 +/- 18 days (mean +/- SEM)]. Urinary excretion of LH, estrone, and pregnanediol was compared to that of nine control breastfeeding mothers. In the control subjects follicular development, as assessed by rises in estrone, was minimal during the first 90 days of the study. Thereafter, phases of estrogen secretion were observed. Ovulation occurred in seven of the nine mothers on one to six occasions; time to first ovulation varied from 90-296 days. In the women taking buserelin, LH and estrone were initially stimulated for 1 and 2 weeks, respectively, then declined to basal levels. No ovulations occurred in the treated group. In six treated mothers only minor fluctuations in estrone were observed during the remainder of agonist treatment. In three subjects more frequent and sustained episodes of estrogen secretion were observed, but in contrast to the controls the rises in estrone were not followed by a typical LH surge or a rise in pregnanediol. Bleeding occurred in eight

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of the nine of the control mothers on one to seven occasions during the study period. The first bleed in five of the mothers was anovular, while other menstrual bleeds occurred in response to falling levels of pregnanediol. Of the mothers taking buserelin, one was amenorrhoeic, and five had only one light bleeding associated with the initial stimulation of estrone. Of the three women with continued fluctuations of estrone, one had three light bleeds, one experienced frequent spotting, while one had regular bleeding. No other side-effects, such as hot flashes or changes in nursing practices, were reported. Our results indicate that LHRH agonist treatment has the potential to be developed as an acceptable method of contraception during the postpartum period. The duration of treatment may be long enough to have a significant effect on maternal-infant well-being without encountering significant problems associated with low estrogen output.

Contracept Fertil Sex (Paris). 1988 Jun;16(6):453-7. Related Articles, Links

[LHRH analogues in female contraception]

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PIP: Since the luteinizing hormone-releasing hormone (LH-RH) has been identified and its mode of action understood, it has become possible to imagine a therapeutic use of long acting, nontoxic analogues. Biochemical modifications of the decapeptide have resulted in the synthesis of potent LH-RH antagonists and agonists. Paradoxically, however, the agonists, devised to induce ovulation, exert an antagonistic action due to a decrease in the number of pituitary LH-RH receptors and to desensitization of the pituitary gland to the decapeptide. These inhibitory effects are associated with the prolonged activity of the analogues, in contrast with the stimulant effects of physiological LH-RH which has a short 1/2-life and is secreted in bursts. These effects have been used in order to inhibit ovulation in female contraception. Preliminary studies clearly indicate a high efficiency of these molecules devoid of metabolic side effects. However, it appears necessary to add sequential progestin therapy on a monthly basis as estradiol levels are those of an early follicular phase. Such a peptide contraception aims at